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RESEARCH AND DEVELOPMENT SERVICES: METHODS DEVELOPMENT

J.J. Mousa, D.H. Powell, R.E. Hall, A.L. Shroads, J.W. Johnson, C.M. Mark, D.M. Victor, and A.C. Moore

Environmental Science and Engineering, Inc.
Post Office Box ESE
Gainesville, Florida 32602

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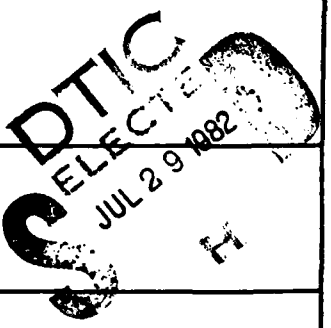

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Method development was conducted to develop analytical methods for eight munition-related compounds in water and soil matrices. Single-analyte and multiple-analyte procedures were developed using GC and HPLC techniques. Precision and accuracy testing of the methods was performed in natural and standard matrices. Detection limits ranged from 1 to 27 ug/L in water matrices and from 0.5 to 4.9 ug/g in soil matrices. An HPLC screening procedure for 14 polynuclear aromatic hydrocarbon (PAH) and 10 nitroaromatic

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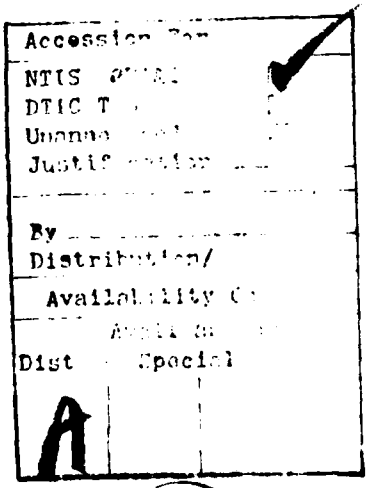
20. compounds was developed which provided quantitative analysis and semiqualitative information by the use of retention times and absorbance ratios at different wavelengths. Single-analyte methods in water and soil were developed for 3,5-dinitroaniline (35DNA); 3,5-dinitrophenol (35DNP); 2,4,6-trinitrobenzaldehyde (ATNBA); HMX; PETN; thiodiglycol (TDGCL); diphenylamine (DPA); and unsymmetrical dimethyl hydrazine (UDMH) (water only). Multiple-analyte methods in water and soil were developed for RDX, HMX, and PETN.



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1.0 BACKGROUND AND OBJECTIVES

Various U.S. Department of Defense (DOD) installations have been the site of chemical/explosive manufacturing and handling operations. Potential migration of toxic or otherwise hazardous materials beyond the borders of military property, contaminated lands, and facilities, and recent pollution control legislation have led to concerted pollution identification and control programs. The U.S. Army Toxic and Hazardous Materials Agency (USATHAMA) has the responsibility of ensuring that the necessary technology and standards are available to define and control the extent of pollution from those operations which have a potential for environmental contamination.

The principal objective of this contract was the development of analytical methods for various munitions-related compounds which could be potential environmental contaminants. For most of these compounds, methods of analysis for environmental soil and water samples were required. The compounds requiring analytical methods development, as originally specified by USATHAMA, are shown in Table 1-1. Based on the results of discussions held with the Project Officer during the project initiation meeting and later developments which occurred during the course of the project, certain compounds were added to this list and others were deleted. Specifically, the single-analyte methods for cyclotrimethylenetrinitramine (RDX) in water and soil matrices were deleted because a method for this analyte had been developed under a previous contract. Thiodiglycol (TDGCL), a potential degradation product of agent H, mustard, was substituted for RDX. No fully documented method for unsymmetrical dimethylhydrazine (UDMH) in soil was developed because of its tendency to rapidly decompose. The desired detection limit range for the developed methods was 1 to 5 parts per billion (ug/L) [micrograms per liter (ug/L)] in water samples and 1 to 5 parts per million (mg/L) [micrograms per gram (ug/g)] in soils. Commonly available chromatographic techniques such as gas chromatography (GC) and high performance liquid chromatography (HPLC) using readily

Table 1-1. Compounds Requiring Methods Development

Number	Compound	Matrix	Required Detection Limit
1	Pentaerythritol tetranitrate (PETN)	Water	1-5 ug/L
2	PETN	Soil	1-5 ug/g
3	Cyclotrimethylenetrinitramine (RDX)*	Water	1-5 ug/L
4	RDX*	Soil	1-5 ug/g
5	Cyclotetramethylenetetranitramine (HMX)	Water	1-5 ug/L
6	HMX	Soil	1-5 ug/g
7	Diphenylamine (DPA)	Soil	1-5 ug/g
8	Unsymmetrical dimethyl hydrazine (UDMH)	Water	1-30 ug/L
9	UDMH**	Soil	1-5 ug/g
10	2,4,6-trinitrobenzaldehyde (ATNBA)	Water	1-5 ug/L
11	ATNBA	Soil	1-5 ug/g
12	3,5-Dinitrophenol (35DNP)	Water	1-5 ug/L
13	35DNP	Soil	1-5 ug/g
14	3,5-Dinitroaniline (35DNA)	Water	1-5 ug/L
15	35DNA	Soil	1-5 ug/g
16	Thiodiglycol (TDGCL)†	Water	1-30 ug/L
17	TDGCL†	Soil	1-5 ug/g

* RDX was deleted from list of compounds because method was already available.
† TDGCL was substituted for RDX.
** Documented soil procedure was not developed because of UDMH instability.

Source: ESE, 1982.

available detectors were the recommended approaches to methods development.

The second objective of this contract was to combine as many of the single-analyte methods as possible into multiple-analyte methods. This effort was directed toward combining methods for analytes with similar chemical and analytical properties into techniques using common methodology.

The final objective of this contract was the development of a quantitative and qualitative HPLC screening procedure for selected nitroaromatics, nitramines and polynuclear aromatic hydrocarbons (PAHs) in water samples. The screening procedure was to be used much like the current U.S. Environmental Protection Agency (EPA) gas chromatography/ mass spectroscopy (GC/MS) screening method for priority pollutants. The HPLC method was to be applicable for the determination of high boiling, nonvolatile compounds which are difficult to analyze by GC. Current state-of-the-art detectors and techniques such as scanning ultraviolet (UV) and UV/fluorescence ratios were to be employed to provide qualitative and quantitative information. Because of their experimental status, technical development, and high instrumentation costs, HPLC/MS techniques were not considered in the screen development. The developed procedure had to have detection limits in the 1- to 5-ug/L range in water for most of the analytes tested. The compounds for which this screen was to be applicable, as originally specified by USATHAMA, are shown in Table 1-2. Because of the unique ionic properties of isopropyl methyl phosphonic acid (IMPA) which make it less amenable to detection and analysis by HPLC and because a specific methodology for its analysis had been developed recently by USATHAMA, IMPA was deleted from the list of potential screen compounds.

Specific documentation and method development requirements were specified by contract for the compounds listed in Table 1-1. First, a single-analyte method had to be developed for each compound in water and

Table 1-2. Compounds Specified for HPLC Screen

Analyte
1. IMPA*
2. RDX
3. Tetryl
4. 35DNP
5. ATNBA
6. Polynuclear Organics (PAHs)
a. Naphthalene, substituted naphthalenes
b. Acenaphthylene, substituted acenaphthylenes
c. Acenaphthene, substituted acenaphthenes
d. Fluorene, substituted fluorenes
e. Anthracene
f. Phenanthrene
g. Pyrene
h. Fluoranthene
i. Naphthacene
j. Triphenylene
k. Chrysene, methyl chrysenes
l. Benzo(a) and benzo(e) pyrene
m. Perylene
n. Dibenzo(a,h)anthracene
o. Indeno(1,2,3)pyrene
p. Coronene

* IMPA was deleted because an analytical method was already developed by USATHAMA.

Source: ESE, 1982.

soil. Testing and documentation of the single-analyte method in standard water, natural water, standard soil, and natural soil were required. Precision, accuracy, and detection limit information was to be provided for each analyte in each matrix. Second, the single-analyte methods were to be combined into as many multiple-analyte methods as possible. These multiple-analyte methods were to be applicable to the analysis of several analytes using common methodology. The multiple-analyte procedure required certification in the test matrices. Finally, for the HPLC screening procedure, documentation in standard and natural water and generation of accuracy, precision, and detection limit data were required.

Other requirements of this project included (1) the implementation of a computerized literature search of domestic and foreign journals for applicable analytical methods, and (2) the development of a project Quality Control (QC) Plan which would specify the laboratory QC procedures to be employed during the project and the specific procedures by which the USATHAMA August 1980 Quality Assurance (QA) Program was to be implemented.

2.0 GENERAL APPROACH

2.1 LITERATURE SEARCH

A computerized literature search of several data bases was conducted to compile analytical methods for the target compounds. A search for analytical methods for PAH analysis by HPLC was also conducted. The literature search included a Chemical Abstracts search from 1972 to 1981 as accessed through the Lockheed Dialog service. Additionally, the Government Reports Annual Index (NTIS) was manually searched for the same years. The computer citations for the analytes are compiled in Appendix A. Pertinent literature references for each target analyte are presented in Sections 3.0 and 4.0. The computerized literature search provided several pertinent references for some analytes, but for most of the target analytes, few applicable analytical methods were found. Several references were found for PAH analysis by HPLC using a variety of detectors such as UV and fluorescence. Review of available research reports prepared for the U.S. Army Armament Research and Development Command regarding environmental fate and chemical and physical properties of munitions-related compounds provided a wealth of valuable information on analytical methods and physical properties for several munition compounds including RDX, HMX, and ATNBA.

2.2 QC PLAN

A project QC Plan was developed which addressed the following items: (1) organization and responsibilities for quality assurance; (2) document control and revisions; (3) training and certification of analysts; (4) method documentation procedures; and (5) analytical systems control, including procedures for instrument calibration and standardization, use of standard reference materials, and notebooks and instrument logbooks. The QC Plan addressed the procedures for development and documentation of analytical methods and the format for submission of analytical methods to USATHAMA. This plan is included as Appendix B.

2.3 SINGLE- AND MULTIPLE-ANALYTE METHODS DEVELOPMENT

Approaches used for the development of single- and multiple-analyte methods are described in Sections 3.1 through 3.7 and 4.1 and 4.2. A complete description of the methods development strategy for the HPLC multiple-analyte screen is presented in Section 4.2.

Proposed Methods Reports were prepared prior to start of methods development work on any analyte or group of analytes. These reports were submitted to USATHAMA for approval of each proposed approach as required by the contract. Each report contained a brief description and justification of the proposed methods development approach. The approved approach served as a basis for the preliminary methods development effort. In most cases, results of preliminary experiments forced minor modifications of the approved approach. Major modifications of the approved approach, which were necessary at times due to the failure of the preliminary approved approach, were presented to USATHAMA for verbal approval before work proceeded.

Single-analyte methods were developed for all analytes except UDMH in soil. Development work on a single-analyte method for PETN in water and soil was combined with work on the multiple-analyte procedure combining RDX, HMX, and PETN. The experimental conditions established for this multiple-analyte method in water and soil were optimum for the analysis of PETN; thus, no separate single-analyte certification process was performed on this compound. Because of its susceptibility to oxidation and rapid loss in aqueous and soil matrices, UDMH presented problems when a soil method development effort was attempted. Kinetic studies performed in soil matrices indicated that under the spiking protocols prescribed by USATHAMA, UDMH could not be recovered quantitatively from a soil matrix. Based on these results, a fully documented soil method for UDMH was not developed, although a qualitative screening procedure is presented in Section 3.5. A list of the single-analyte and multiple-analyte methods developed and the detection limits in standard and natural water and soil are given in Table 2-1.

Table 2-1. Developed Single- and Multiple-Analyte Methods

Analyte(s)	Matrix	Detection Limit (Natural Media)	Detection Limit (Standard Media)
Single-Analyte Methods			
HMX	Water	0.97 ug/L	2.9 ug/L
	Soil	4.9 ug/g	4.8 ug/g
PETN*	Water	4.5 ug/L	3.4 ug/L
	Soil	2.3 ug/g	2.4 ug/g
35DNP	Water	4.5 ug/L	3.8 ug/L
	Soil	1.7 ug/g	1.9 ug/g
35DNA	Water	3.0 ug/L	1.0 ug/L
	Soil	1.1 ug/g	0.5 ug/g
ATNBA	Water	2.2 ug/L	3.3 ug/L
	Soil	3.5 ug/g	2.0 ug/g
UDMH	Water	11 ug/L	16 ug/L
TDGCL	Water	119 ug/L	27 ug/L
	Soil	2.2 ug/g	4.1 ug/g
Diphenylamine	Soil	1.6 ug/g	1.5 ug/g
Multiple-Analyte Methods			
HMX, RDX, PETN	Water	2.3, 4.1, 4.5 ug/L†	1.8, 1.5, 3.4 ug/L†
		4.1, 2.7, 2.3 ug/g	4.6, 1.9, 2.4 ug/g
HPLC Screen (24 compounds)	Water	0.3 to 35 ug/L	

* Same as multiple-analyte method.
† HMX, RDX, and PETN, respectively.

Source: ESE, 1982.

The strategy for development of the analytical methods consisted of:

1. Selection and evaluation of an extraction/concentration technique;
2. Development of a cleanup procedure, if required; and
3. Establishment of appropriate selective and sensitive GC or HPLC conditions for analysis of the extracts.

Extraction techniques which had been used successfully for certain analytes were the primary test extraction techniques used for the other analytes. The cleanup strategy relied heavily on the use of silica-gel and Florisil® Sep-Pak® chromatography, sequential elution with solvents of low-to-medium polarity for elution of possible interferences, and elution with solvents of medium-to-high polarity to isolate the analytes of interest. HPLC techniques were favored over GC analysis because of the high boiling points, highly polar chemical nature, and relative thermal instability of most of the analytes.

Examination of the chemical properties and structures of the single analytes in Table 1-1 revealed only one group of compounds which appeared suitable for inclusion in a multiple-analyte method. HMX and PETN had similar nonaromatic, nitrated, and chemical structures and both could be extracted from water by methylene chloride. In addition, HMX and PETN had similar HPLC behavior; therefore, they were grouped together in one multiple-analyte approach. Although RDX was deleted from the list of target analytes, the unlikely presence of this compound in environmental samples contaminated with HMX required that the developed procedure for HMX analysis be capable of separating RDX from HMX, thereby providing quantitation of both explosives and preventing possible interference in the specific analysis of HMX in real samples. RDX was therefore included in the standard solutions used in documenting and developing this method. Although only one multiple-analyte procedure was developed, the developed HPLC screening procedure described in Section 4.2 is a multiple-analyte procedure capable of analyzing 10 nitroaromatic compounds in a single injection.

After the single- and multiple-analyte analytical methods were developed, data which characterize the method or demonstrate the ESE laboratory's ability to perform the method were submitted to the USATHAMA Chemistry Group for review. These data included estimates of the standard deviation, percent inaccuracy, and percent imprecision of the method calculated from spiking experiments into standard and natural matrices. The standard deviation, s, was calculated at each target-spiked concentration according to the following formula:

$$\text{Standard deviation} = s = \left(\frac{\Sigma X_i^2 - \frac{(\Sigma X_i)^2}{n}}{n-1} \right)^{1/2}$$

where: X_i = the ith found concentration,
n = the total number of X values, and
Σ = summation from i = i to i = n.

The percent inaccuracy was calculated at each target concentration according to the following formula:

$$\text{Percent inaccuracy} = \frac{\bar{x} - TC}{TC} \times 100$$

where: $\bar{x}$ = average found concentration at the particular TC, and
TC = target concentration.

The percent imprecision was calculated at each target concentration according to the following formula:

$$\text{Percent imprecision} = \frac{s}{\bar{x}} \times 100$$

where: s = standard deviation, and
$\bar{x}$ = average found concentration at the particular target concentration.

In addition, the detection limit, precision, and accuracy of the developed method were determined from the least-squares regression line of the found-versus-target concentration data. The detection limit was

calculated using the method of Hubaux and Vos (1970), described as follows.

The detection limit was derived from the following assumptions: (1) the relationship between the found concentration and target concentration was linear, (2) the variance about the least-squares linear regression line was homogeneous over the tested concentration range, and (3) the distribution of found concentrations for a given target concentration was a normal distribution. Based on these assumptions, the least-squares linear regression line of the formula:

$$Y = Yo + bx$$

was determined where

Yo = Y-axis (found concentration) intercept,

b = slope of the line, and

x = target concentration.

The confidence limits about the regression line were determined for the upper confidence limit as follows:

$$y = yo + bx + S_{y.x}\, t \left(\frac{1}{n} + \frac{1}{N} + \frac{(x-\bar{x})^2}{\sum (x-\bar{x})^2} \right)^{1/2}$$

and for the lower confidence limit as follows:

$$y = yo + bx - S_{y.x}\, t \left(\frac{1}{n} + \frac{1}{N} + \frac{(x-\bar{x})^2}{\sum (x-\bar{x})^2} \right)^{1/2}$$

where:

$$S_{y.x} = \left(\frac{\sum (y_i-\bar{y})^2 - b^2 \left(\sum x_i-\bar{x}\right)^2}{N-2} \right)^{1/2}$$

t = student's t for 95-percent confidence and N-2 degrees of freedom,

n = number of replicates of each target concentration, and

N = number of target concentrations.

The detection limit is the value of X corresponding to a point on the lower confidence limit curve where the value of y equals the value of y on the upper confidence limit curve at X = 0.

The slope, b, of the least-squares regression line of the plot of found-versus-target concentrations is a measure of the accuracy of the method. The slope should be plus one (+1.0) for 100-percent recovery for the complete analytical method. Experimental values will deviate from this expected value.

The standard error of the estimate ($S_{y.x}$) for the least-squares regression line of found-versus-target concentrations is a measure of the precision of the analytical method.

2.4 STANDARDS AND STANDARD MATRICES

Standard and spiking solutions of the test analytes were prepared from Standard Analytical Reference Material (SARM) and interim SARMs received from USATHAMA, or from commercially available materials if SARMS were unavailable. The origin of each of the standards used in this contract is shown in Tables 2-2 and 2-3.

Documentation of the analytical methods was performed in two water and two soil matrices. All methods were documented in standard water. Standard water for organic analytes, according to the USATHAMA August 1980 QA Plan, is laboratory-distilled water containing 100 mg/L of $SO_4^{=}$ and 100 mg/L of Cl^{-}. The natural water used is this study was obtained from a freshwater lake (Orange Lake) located in Alachua County, Florida. The water had a greenish-yellow tint due to a large phytoplankton population.

Because of the large volume of natural water used in the documentation process, several batches of Orange Lake water were used during this project. No significant differences between batches were noted in the level of background interferences encountered during methods

Table 2-2. Analytical Standards Supplied by the Government

Compound	U.S. Army I.D. No.	Lot No.	Purity (%)	Distribution Date
SARMS				
RDX	PA 665	HOL 475-1	99.82	7/23/81
PETN	PA 628	Battelle Lab	--*	5/04/81
35DNP	PA 625	1235	99.95	4/24/81
35DNA	PA 416	2225	99.86 MOL	8/02/80
DPA	PA 414	1132	99.83 MOL	8/02/80
Tetryl	PA 666	2714	99.70	7/23/81
1,3-Dinitrobenzene (13DNB)	PA 667	2250	99.99	7/20/81
2,4,6-Trinitrotoluene (246TNT)	PA 273	268	99.80	1/11/80
2,6-Dinitrotoluene (26DNT)	PA 661	31640-A	99.62	7/23/81
2,4-Dinitrotoluene (24DNT)	PA 626	28493-A	99.64	5/05/81
CONDITIONAL SARMS				
HMX	PA 664	--	--	7/23/81
ATNBA	PA 659	--	--	7/23/81
1,3,5-Trinitrobenzene (135TNB)	PA 660	1-157-1	--	7/23/81
PROVISIONAL SARMS				
TDGCL	PA 418	1232	--	--

* Information not available.

Source: ESE, 1982.

Table 2-3. Commercially Obtained Analytical Standards

Compound	Catalog Number	Lot Number	Purity (%)
UDMH	Eastman 7053	--*	98 minimum
Acenaphthene	Aldrich A10-4	LB072967	99
Acenaphthylene	Aldrich A80-5	AB091737	99
Phenanthrene	Aldrich P1,140-9	LB091577	98+
Indeno(1,2,3-cd)pyrene	Chem Service FL-82	5-19	99
Benzo(b)fluoranthene	Chem Service F74/A2119F	3-90	99
Benzo(k)fluoranthene	Chem Service F75/A2122F	5-18G	97.3
Dibenzo(a,h)anthracene (1,2,5,6)Dibenzanthracene	Aldrich D3,140-D	122257	97
Naphthalene	Chem Service 0-787	--	--*
Anthracene	Aldrich 14,106-2	JD010897	99.9+
Fluoranthene	Aldrich F80-7	LB092977	98
Pyrene	Aldrich 18,551-5	AB090267	99+
Chrysene	Aldrich C8,000-8	AB090967	95
Benzo(a)pyrene	RFR, Corp. RAH-10	--	99

* Information not available.

Source: ESE, 1982.

development. One batch of the natural test water contained approximately 73 milligrams per liter (mg/L) total solids and a total organic carbon level of 12.8 mg/L. The term "standard" soil was applied to a clean Alabama red clay soil obtained from the Alabama Army Ammunition Plant (AAAP) in 1979. This soil had been used as the standard test matrix for the documentation of methods for nitroaromatics, organic bases, and various inorganic species during a previous environmental survey of AAAP. This clay matrix would be fairly representative of most soils in the United States which are, to some extent, clay soils. The other soil matrix used to document the developed methods was the "natural" soil which consisted of a builder's soil obtained in Gainesville, Florida. This soil was sandy in appearance, but also contained visible organic matter. All soils were air-dried and sieved through a 30-mesh sieve before use in the documentation.

3.0 SINGLE-ANALYTE METHODS DEVELOPMENT

Approaches used for the development of an optimized single-analyte procedure for each analyte are described in the following sections. A brief review of the pertinent chemical and physical properties of each analyte, the results of the literature search for analytical methods, a summary of the preliminary and method optimization experimental results, and justification for the selection of the final methods development approach are included in the following sections.

3.1 3,5-DINITROPHENOL (35DNP)

3.1.1 Physical and Chemical Properties

Some of the physical and chemical properties of 35DNP are listed in Table 3-1. The chemical structure is presented in Figure 3-1. 35DNP undergoes normal phenolic reactions such as acid dissociation (Chantooni and Kolthaff, 1976). Rochester and Wilson (1976) reported the acid dissociation constant as 1.9×10^{-7}. 35DNP has been reported to be soluble in water, alcohol, ether, benzene, and chloroform. No references were found on either the stability or UV spectrum of 35DNP. The UV spectrum obtained for 35DNP in a 1% acetic acid/water medium for this contract is presented in Figure 3-2. The spectrum reveals absorbance maxima at 254 nanometers (nm) and 344 nm. The 344-nm absorbance is 5.1 times weaker than the 254-nm absorbance. A typical end absorbance at wavelengths below 220 nm is evident.

3.1.2 Results of Literature Search for Analytical Methods

The computerized literature search provided little specific information on analytical methods for 35DNP. Investigations into specific analytical techniques for nitrophenols showed an extreme scarcity of information in this area.

Hrivnak et al. (1969) tested GC on polyester-type GC columns with no success. EPA published a method for phenols, Method 604, in the Federal Register (U.S. Environmental Protection Agency, 1979a). This is a GC

Table 3-1. Alternate Nomenclature and Physical and Chemical Properties of 35DNP

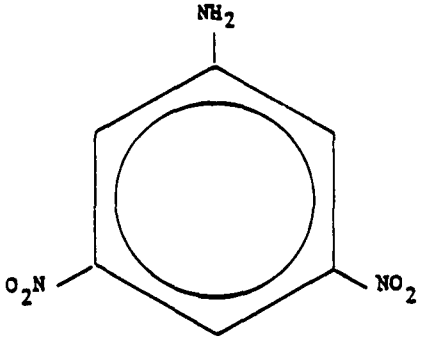
A. ALTERNATE NOMENCLATURE AND CHEMICAL ABSTRACT SERVICE (CAS) REGISTRY NUMBER

Analyte	Alternate Nomenclature	CAS Registry Number
35DNP	None	586-11-8

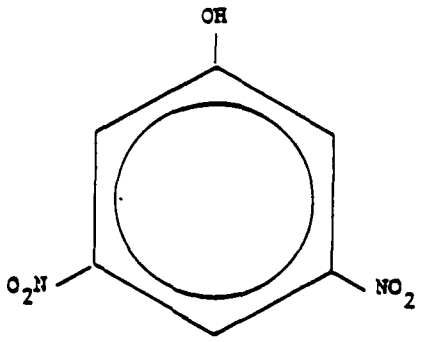
B. PHYSICAL AND CHEMICAL PROPERTIES

Analyte	Formula	Melting Point (°C)	Acid Dissociation Constant
35DNP	$C_6H_3O_5N_2$	126	$pk_a = 6.7$

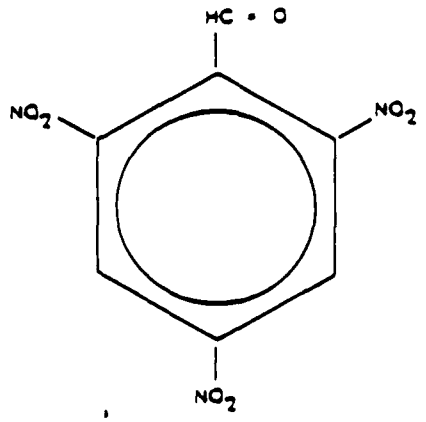
Source: ESE, 1982.



3,5-Dinitroaniline (35DNA)



3,5-Dinitrophenol (35DNP)



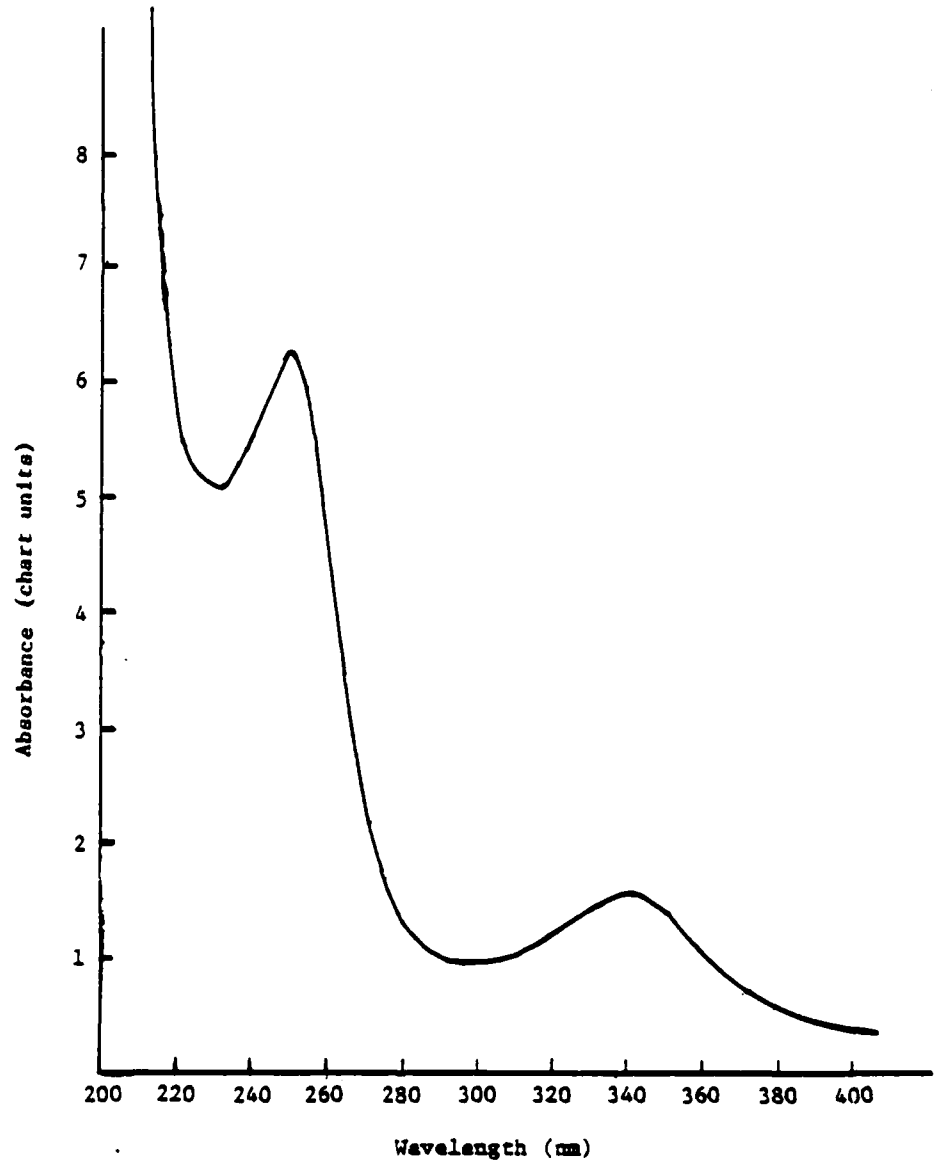
2,4,6-Trinitrobenzaldehyde (ATNBA)

SOURCE: ESE, 1982.

Figure 3-1

CHEMICAL STRUCTURES OF 35DNA, 35DNP, AND ATNBA

Prepared for:
U.S. Army Toxic and Hazardous Materials Agency
Aberdeen Proving Ground, Maryland



SOURCE: ESE, 1982.

Figure 3-2

UV ABSORPTION SPECTRUM OF 35DNP IN WATER (1% ACETIC ACID)

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method using flame ionization detection, or a derivatization followed by electron capture (EC) detection. Method 604 is applicable to the analysis of o-nitrophenol, p-nitrophenol, and dinitro-o-cresols. But experience with this method at ESE has shown that the stationary phases used with this method are not especially stable.

The recent Analytical Chemistry Application Reviews (1981) lists numerous GC and HPLC methods for phenols in water involving techniques such as derivatization, fluorescence, and electrochemical detection. None of these techniques were specifically applicable to nitrophenols but concentrated on chlorophenols and cresols. The general trend of the articles in the literature indicated that HPLC analysis would be the most viable technique for nitrophenol analysis.

3.1.3 Preliminary Experiments and Method Optimization--Water

Historically, phenols have been analyzed at trace levels by derivatization followed by gas chromatographic detection. However, HPLC has become an alternative and successful approach for phenol analysis and detection. Because of the potential problems associated with a derivatization GC method, an HPLC approach was favored in this work.

HPLC separation conditions for 35DNP were initially determined on an Ultrasphere-ODS column (4.6-mm ID by 25 cm, 5-um particle size). The initial mobile phase composition tested was 70% methanol/30%, 0.1 M potassium dihydrogen phosphate buffer adjusted to a pH of 3. However, the aqueous buffer exhibited a tendency to precipitate in the chromatographic system and, therefore, a 70% methanol/30% water mobile phase containing 0.03 M phosphoric acid was substituted. Under these conditions and at a flow rate of 1 milliliter per minute (ml/min), the retention time for 35DNP was 5.35 minutes. The minimum detectable amount of 35DNP with 254-nm UV detection was 20 nanograms (ng).

Using this column system, other potential detectors were evaluated for applicability to 35DNP analysis. The purpose of these investigations

was to determine if a more selective detector could be used than the standard 254-nm UV detector. The response of 35DNP to oxidative electrochemical detection using a glassy-carbon electrode was investigated. This approach seemed to have potential due to the extreme sensitivity with which chlorophenol compounds had been detected during previous experiments and due to reports of its successful use in the literature by Lores, Edgerton, and Moseman (1981). At an applied potential of -1.15 volts, the minimum detectable amount was 500 ng, which was not very sensitive. From Hammett linear free energy considerations, nitrosubstituent groups on phenols would be expected to deactivate the compound with respect to oxidation (Suatoni et al., 1961), and thus the low sensitivity to electrochemical detection was not surprising. Fluorescence detection was also briefly examined, but the sensitivity was limited, as expected for nitrosubstituted phenols (Ogan and Katz, 1979).

Due to the poor response of 35DNP to both electrochemical and fluorescence detection, UV absorbance detection at 254 nm was selected for further work. Under the chromatographic conditions evaluated for 35DNP, substantial background interferences were found at this wavelength during later analyses of natural water extracts, which required the availability of better detector selectivity. Greater selectivity and freedom from interferences were achieved by using a 340-nm filter in the fixed-wavelength UV detector and using the absorbance maximum at 344 nm as the band for detection of 35DNP. Although, the sensitivity at this wavelength was five times less than at 254 nm, a larger injection volume of 250 microliters (ul) and concentration of the final sample extract to a smaller final volume [2 milliliters (ml) instead of 5 ml] compensated for this loss of sensitivity. Use of the 340-nm filter kit supplied with the Altex Model 153 fixed-wavelength detector caused an apparent broadening of the peak shape for 35DNP. The reason for this broadening was not readily apparent. This effect did not occur when a continuously variable-wavelength UV detector was used for the analysis. However, this peak

broadening had no adverse effect on the ability to develop and document a method for 35DNP.

A tentative approach for the extraction and concentration of 35DNP from water was selected. This approach used a base/neutral (pH = 12), methylene chloride extraction for removal of interferences, followed by an acidic extraction at pH = 3 to isolate the 35DNP and other phenolics. The initial base/neutral extraction with methylene chloride provides some sample cleanup because most neutral and basic organic interferences are removed. The 35DNP and other phenolics remain in the aqueous basic solution during this extraction because of their acidic properties. The next step involved acidification of the remaining aqueous phase to a pH of 3, followed by methylene chloride extraction of the 35DNP from the acidic solution. The extract is reduced in volume using the Kuderna-Danish (K-D) concentrative evaporation technique and the solvent exchanged to methanol. The methanol extract is diluted with water for injection into the HPLC.

To test the extractability of 35DNP from acidic solution, methylene chloride extractions of spiked waters at a pH of 3 were monitored by direct injection of the water phase after extraction. It was found that the addition of 100 grams of sodium chloride per 900 ml of sample was necessary to achieve good recoveries. Waters spiked at 100 ug/L of 35DNP were extracted and concentrated in the procedure described in the previous paragraph, and recoveries of 71 and 68 percent were obtained. Improvement of these resources was attempted because recoveries at lower spiking levels (1 to 10 ug/L) were expected to be less than those at the 100-ug/L level.

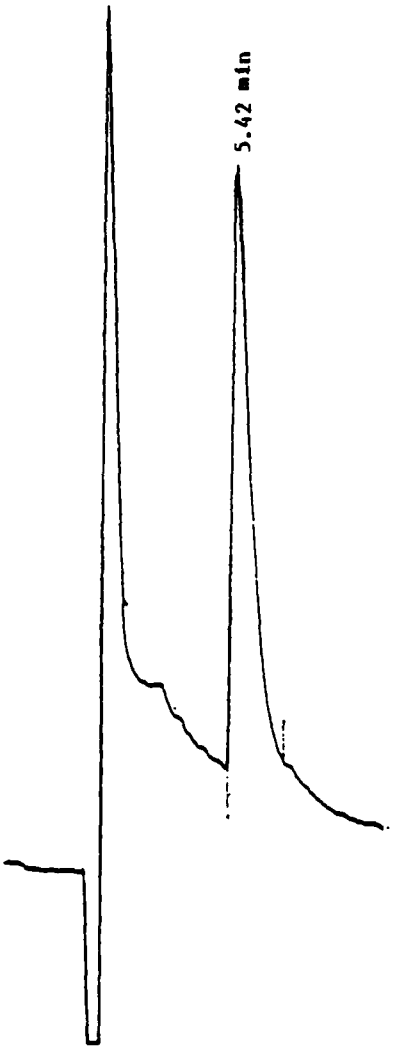
In their experiments with phenol analysis, Hrivnak et al. (1969) acid-washed their glassware, any sodium sulfate used for drying of extracts, and glass wool used in the extraction procedure. It was felt that the preliminary recovery for 35DNP could be improved if these techniques were implemented. These modifications were performed, and

recoveries at the 100-ug/L level were 109 and 88 percent. Because the extraction and concentration procedures now appeared acceptable, attention was turned to the development of a cleanup method for the extract.

Methylene chloride was found to elute 35DNP from silica-gel Sep-Paks®, but not from Florisil® Sep-Paks®. The behavior of 35DNP on Florisil® Sep-Paks® could be used to good advantage in developing a cleanup procedure because the analyte could be loaded on the cartridge in methylene chloride, and the methylene chloride, which would contain many nonpolar and moderately polar interferences, could be discarded. The Florisil® could then be eluted with 5% methanol in methylene chloride, a slightly more polar solvent, to remove 35DNP. This approach proved successful and was incorporated into the method.

Because the extraction and chromatography procedures seemed to be optimized for 35DNP analysis, documentation of the method in standard water was attempted. Low recoveries (50 to 60%) were obtained for samples which were allowed to sit overnight in the K-D flasks prior to evaporation. Higher recoveries were obtained when the K-D glassware was acid-washed with a 50% concentrated hydrogen chloride/50% water solution, probably due to deactivation of active basic sites on the glassware. A 96-percent recovery was obtained from a 100-ug/L level spike which sat overnight in the acid-washed glassware. With these modifications, the method for 35DNP in water appeared to be the optimum method, and documentation in standard and natural waters commenced with no further problems. A chromatogram of 35DNP in natural water spiked at 5.42 ug/L is presented in Figure 3-3.

Method Documentation--Quantitative method documentation was performed in accordance with the project QC Plan. The analytes were spiked into the standard and natural waters at 0, 0.5 DL, 1 DL, 2 DL, 5 DL, and 10 DL (DL = detection limit), where DL is 2.7 ug/L. The entire analysis was performed with these spiked samples. Replicates were analyzed at the



Column: Ultrasphere-ODS (4.6-mm ID x 250 mm, 5 um)
Detector: Altex Model 153 fixed-wavelength detector
(λ = 340 nm)
Flow Rate: 1 ml/min
Mobile Phase: 70% methanol/30% water (0.03M H_3PO_4)
Injection Volume: 250 ul, fixed-loop

SOURCE: ESE, 1982.

Figure 3-3

CHROMATOGRAM OF 35DNP IN NATURAL WATER SPIKED AT 5.42 ug/L

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six spiking levels on each of 4 separate days. The detection limit of the method was calculated by applying the Hubaux and Vos (1970) method to the found-versus-target concentration data. The precision and accuracy of the method were determined from the standard estimate of the error ($S_{y.x}$) and slope, respectively, of the least-squares regression line of the found-versus-target concentration data. The documented method and data are presented in Appendix C. The accuracy of the method over the entire spiking range (1.4 to 27 ug/L) was 76 percent for natural water. The calculated accuracy was lower than that expected from the earlier recovery data obtained at 100 ug/L but considering the lower spiking levels, was not entirely out of line. The Hubaux and Vos detection limit was 3.8 ug/L in standard water and 4.5 ug/L in natural water. The average percent imprecision of the method over the spiking range in natural water was 13 percent.

3.1.4 Preliminary Experiments and Method Optimization--Soil

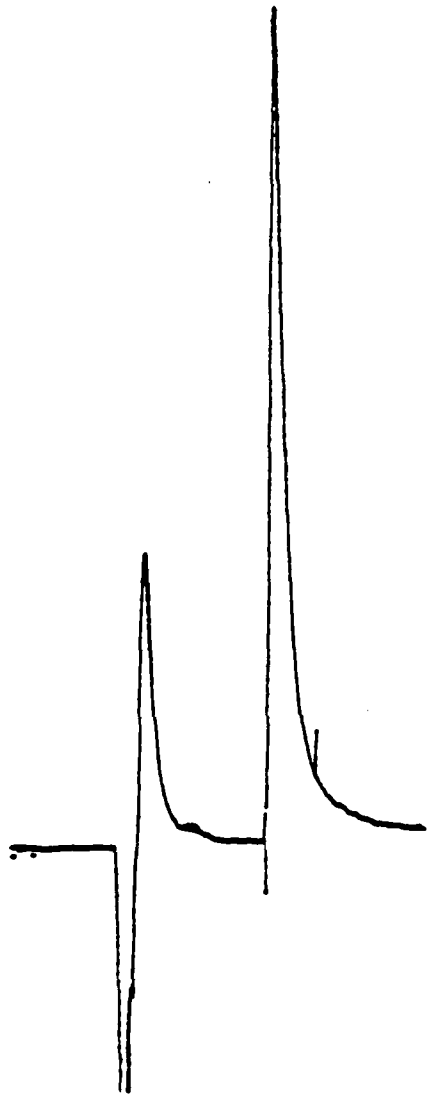
The initial approach to development of a soil method for 35DNP was to modify the completely developed water method. Water extraction of the soil was implemented as a first step for soil analysis because 35DNP is moderately soluble in water. The 35DNP water method could then be applied to analyze the extract.

Water extraction of the soil was tested. In the soil extraction procedure, a 20-gram aliquot of soil was shaken vigorously with 35 ml of water at a pH of 7 for several minutes in a 50-ml glass centrifuge tube. After each extraction, the mixture was centrifuged to settle the soil particles and the water phase was decanted into a separatory funnel. Natural soil (20 grams) was spiked at the 3-ug/g level, and the water extract was monitored directly by direct injection onto the HPLC column. The observed recoveries were as follows:

Extraction	Volume (ml)	% Recovery Natural Soil
1st	35	76.9
2nd	35	12.9
	Total	89.8

Because these preliminary recoveries were good, evaluation of the complete method commenced including aqueous extraction of the soil and analysis of the water extract using documented water procedure. Natural and standard soil samples were spiked at 3 ug/g and analyzed with the proposed method. After vigorous extraction with water, the standard soil sample, a red clay, was centrifuged at 1,000 revolutions per minute (rpm) for 5 minutes to settle the clay particles and leave a clean, aqueous extract. The sample was centrifuged at less than 3,000 rpm to avoid severe compaction of the soil. Compaction made the clay difficult to resuspend for the next extraction. The natural soil, a sandy soil with some black humus matter, could be settled properly from the aqueous extract only by centrifuging at 3,000 rpm for 15 minutes. Otherwise, the suspended solids would cause emulsion problems in the next procedure step (methylene chloride extraction). The recoveries in these tests ranged from 79 to 68 percent. A chromatogram of 35DNP in an extract of a natural soil sample spiked at 1.22 ug/g is presented in Figure 3-4.

Method Documentation--Quantitative method documentation was performed in accordance with the project QC Plan. The analytes were spiked into standard and natural soils at 0, 0.5 DL, 1 DL, 2 DL, 5 DL, and 10 DL, where DL is 1.22 ug/g. These spiked samples were analyzed with the entire process. Replicates were analyzed at the six spiking levels on each of 4 separate days. The detection limit was calculated using the Hubaux and Vos procedure from the target-versus-found concentration data. Precision and accuracy data were calculated from the standard error of the estimate ($S_{y.x}$) and the slope of the least-squares regression line of the found-versus-target data. The documented method and data are presented in Appendix C. The Hubaux and Vos (1970) detection limit for this method is 1.7 ug/g in natural soil and 1.9 ug/g in standard soil. The accuracy of the method over the spiking range (0.61 to 12.2 ug/g) was 79 percent for natural soil, and the average percent imprecision was 17 percent. No matrix interferences were observed during method documentation.



Column: Ultrasphere-ODS (4.6-mm ID x 25 cm, 5 um)
Detector: Altex Model 153 fixed-wavelength detector
(λ = 340 nm)
Flow Rate: 1 ml/min
Mobile Phase: 70% methanol/30% water (0.03M H_3PO_4)
Injection Volume: 250 ul, fixed-loop

SOURCE: ESE, 1982.

Figure 3-4

CHROMATOGRAM OF 35DNP IN NATURAL SOIL SPIKED AT 1.22 ug/g

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3.2 3,5-DINITROANILINE (35DNA)

3.2.1 Physical and Chemical Properties

Some of the physical and chemical properties and alternate nomenclature for 3,5-dinitroaniline (35DNA) are listed in Table 3-2. The chemical structure of 35DNA is presented in Figure 3-1. 35DNA is a weak aromatic base. Based on thermodynamic parameters for anilines, as determined at different temperatures by Bolton and Hall (1970), the pK_a of the 3,5-dinitroanilinium ion was determined to be 0.23. Therefore, 35DNA should exist as a free nonionized molecule at a pH greater than 2. The UV spectrum of 35DNA was determined experimentally and was in agreement with the Sadtler Index spectra (Sadtler Research Laboratories, Inc., 1966). The absorbance maxima observed in the spectra and the relative intensities as determined during this experiment are:

Wavelength (nm)	Relative Absorbance
227	10.0
255	4.9
395	1.0

3.2.2 Results of Literature Search for Analytical Methods

Literature references were extremely scarce in the area of specific analytical methods for the determination of 35DNA. A GC/EC method for the determination of certain DNA pesticides was reported in EPA Method 627 (U.S. Environmental Protection Agency, 1979b). However, these compounds were dinitroaniline derivatives, not free anilines.

Related compounds such as benzidine, dichlorobenzidine, and diphenylhydrazine have been determined directly in water at the 1-ug/L level by Riggin and Howard (1979) using HPLC with electrochemical detection. These compounds contain free amino functional groups as does 35DNA. Aromatic amines have also been determined by derivatization techniques and GC using various derivatizing agents such as trifluoroacetic acid, heptafluorobutyric acid, and other halogenated acetylating agents.

Table 3-2. Alternate Nomenclature and Physical and Chemical Properties of 35DNA

A. ALTERNATE NOMENCLATURE AND CAS REGISTRY NUMBER

Analyte	Alternate Nomenclature	CAS Registry Number
35DNA	1-Amino-3,5-dinitrobenzene 3,5-Dinitrobenzenamine	618-87-1

B. PHYSICAL AND CHEMICAL PROPERTIES

Analyte	Formula	Melting Point (°C)	Acid Dissociation Constant
35DNA	$C_6H_5O_4N_3$	160 - 162	pk_a = 0.23

Source: ESE, 1982.

3.2.3 Preliminary Experiments and Method Optimization--Water

Preliminary investigations concentrated on finding optimum conditions for the analysis of 35DNA using HPLC or GC. Various detector systems were also investigated. 35DNA was initially analyzed using HPLC conditions which had been developed for 35DNP. These conditions were: Ultrasphere-ODS column (5-um particle size, 25 cm by 4.6-mm ID); flow rate of 1 ml/min; and mobile phase of 70% methanol/30% water (pH 3, 0.1 M phosphate buffer). The UV monitoring wavelength was 254 nm. The retention times under these conditions were 5.0 minutes for 35DNA and 5.5 minutes for 35DNP, which seemed suitable for further development. The applicability of electrochemical and fluorescence detection systems was studied briefly, but poor response was found using both the electrochemical detector in the oxidative mode and the fluorescence detector. Further evaluation of the UV detector was pursued. The phosphate buffer was eliminated from the HPLC mobile phase conditions for 35DNA analysis in later experiments because it was necessary for electrochemical detection but not for UV detection.

Direct analysis of 35DNA by a GC/EC method without derivatization was studied briefly, without success. A 6-foot, 5-percent SP-2401 DB on 100/120-mesh Supelcoport column (a specially deactivated column designed for analysis of anilines) was evaluated. No elution of the 35DNA peak was observed on this column even at temperatures up to 220°C (the maximum allowed column temperature for this particular phase), presumably due to adsorption on the column. Because of the limited success with these GC/EC approaches, HPLC with UV detection seemed the more promising approach, and GC/EC methods were not pursued further.

Work was initiated on the development of an extraction and concentration procedure for 35DNA from water samples. Methylene chloride was the extracting solvent of choice because of its convenient solvent properties such as its low boiling point and moderately polar chemical nature. Although specific solubility data were not readily available for 35DNA, other similar nitroanilines were known to be very soluble in

methylene chloride. An initial experiment using methylene chloride extraction of a spiked water sample was performed. Standard water was spiked at 230 ug/L with 35DNA and extracted with three 60-ml aliquots of methylene chloride. The aliquots were dried by passing them through a funnel containing approximately 10 grams of anhydrous sodium sulfate into a K-D apparatus. The combined extracts were concentrated and solvent-exchanged to methanol. Analysis by HPLC of duplicate experiments gave recoveries of 99.2 and 99.8 percent.

Chromatographic interferences in the form of high chromatographic backgrounds had been found in earlier methods development work for 35DNP in natural water using UV detection at 254 nm. Because the chromatographic conditions for the analysis of 35DNA and 35DNP were similar, detector conditions to reduce possible chromatographic interferences in 35DNA analysis were required. A monitoring wavelength of 395 nm was used instead of 254 nm. Although the response at this higher wavelength for 35DNA was five times less than the response at 254 nm, sufficient sensitivity existed for analysis if larger injection volumes of 250 ul were used. The primary advantage of this wavelength (395-nm) is that potential interferences from the sample matrix are greatly reduced.

Another factor which influenced the level of chromatographic interferences was the extraction pH. 35DNA was found to be extractable from water at all pH values from 2 to 12. The selection of an optimum extraction pH would be influenced by the level of potential interfering materials which were co-extracted at the particular pH values used. Unspiked natural water samples were extracted with methylene chloride at pHs of 4 and 12. The extracts were concentrated, exchanged to methanol solvent, and analyzed by HPLC at several wavelengths. The level of background interference was measured in chart units of pen deflection at the retention time of 35DNA. The results are presented in Table 3-3. The data indicate that optimum conditions for minimization of interferences are extraction at pH 12 and HPLC analysis using a 395-nm monitoring wavelength.

Table 3-3. Variation of Extracted Natural Water Background with pH and Monitoring Wavelength

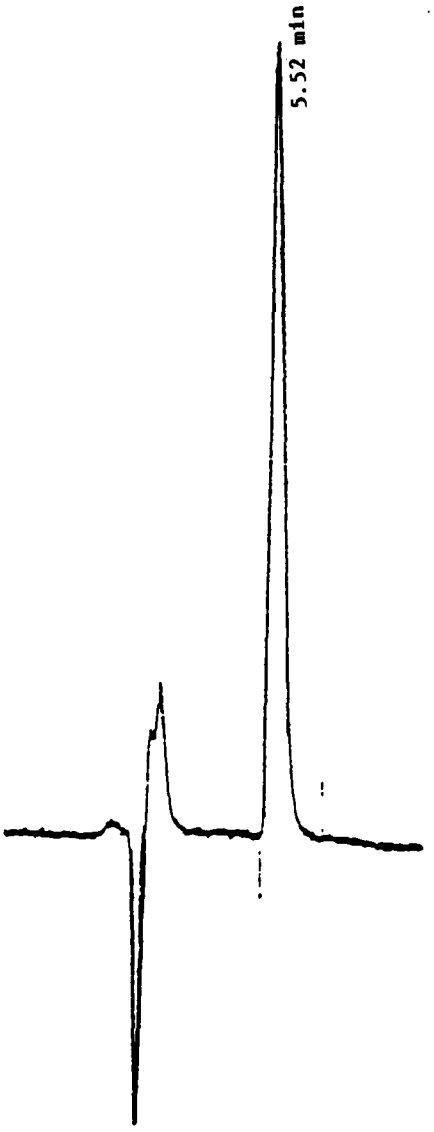
Extraction pH	Wavelength (nm)	Background Response in Chart Units (mm) at 5.5-min Retention Time
4	254	832
4	340	128
4	395	6
12	254	128
12	340	16
12	395	0

Source: ESE, 1982.

Because the initial extraction experiments had resulted in excellent recoveries, possible cleanup procedures were investigated. Because the polarities of 35DNA and 35DNP were similar, the Sep-Pak® cleanup procedure developed for 35DNP was evaluated for applicability to 35DNA. Methylene chloride spiked with 198 ug of 35DNA was passed through a Florisil® Sep-Pak® which was subsequently eluted with 3 ml of 5% methanol in methylene chloride. The recovery was 98.9 percent, and this cleanup step was incorporated into the proposed method.

One set of natural water samples spiked at 0.5 to 10 ug/L was analyzed using the completely developed method which entailed using a pH-12 methylene chloride extraction, K-D concentration, Florisil® Sep-Pak® cleanup, solvent-exchange to methanol, and HPLC analysis with UV detection at 395 nm. The recoveries for this set ranged from 82 to 99 percent. In view of these excellent results, full documentation using standard and natural waters proceeded. A chromatogram of 3,5 DNA in natural water spiked at 2.33 ug/L is presented in Figure 3-5.

Method Documentation--Quantitative method documentation was performed in accordance with the project QC Plan. The documented method and data are presented in Appendix C. The analytes were spiked into standard water and natural water at 0, 0.5 DL, 1 DL, 2 DL, 5 DL, and 10 DL, where DL is 1.17 ug/L. These spiked samples were analyzed with the entire procedure. Replicates were analyzed at the six spiking levels on each of 4 separate days. The detection limit was calculated from the found-versus-target concentration data using the method of Hubaux and Vos (1970). The detection limit was 1.0 ug/L in standard water and 3 ug/L in natural water. The slope and standard error of the estimate (Sy.x) of the least-squares regression line of the found-versus-target concentration data were used to calculate the accuracy and precision of the method. The accuracy of the method over the spiking concentration range (0.58 to 11.7 ug/L) was 83 percent in natural water. The average percent imprecision of the method over the spiking range was 15 percent.



Column: Ultrasphere-ODS (4.6-mm ID x 25 cm, 5 um)
Detector: Perkin-Elmer LC-75 variable-wavelength detector (λ = 395 nm)
Flow Rate: 1 ml/min
Mobile Phase: 70% methanol/30% water
Injection Volume: 250 ul, fixed-loop

SOURCE: ESE, 1982.

Figure 3-5

CHROMATOGRAM OF 35DNA IN NATURAL WATER SPIKED AT 2.33 ug/L

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3.2.4 Preliminary Experiments and Method Optimization--Soil

A modification of the completed water method to include a soil extraction step was the logical first step in the soil method development. Water was to be used as the extracting solvent for soil extraction because methylene chloride may have a tendency to extract interferences. The aqueous soil extract would be adjusted to a pH of 12, extracted with methylene chloride, K-D concentrated, cleaned with a Florisil® Sep-Pak®, and analyzed by HPLC with UV detection at 395 nm.

Initial tests of the water extraction of the soil were performed by spiking natural and standard soils (20 grams each) at 2.6 mg/L (ug/g) and extracting sequentially with three 35-ml portions of water in a 50-ml, capped centrifuge tube. Each portion was monitored for extraction efficiency by direct injection of the aqueous extract on the HPLC column. The recoveries observed were:

Extraction Aliquot	Natural Soil (%)	Standard Soil (%)
1st	54.9	72.9
2nd	10.8	15.0
3rd	4.3	4.8
Total	70.0	92.7

The recoveries achieved were acceptable, and the water extraction approach for the analysis of soils was pursued.

Testing of the complete tentative soil procedure was begun. Standard soil was spiked at 0, 0.5 DL, 1 DL, 2 DL, 5 DL, 10 DL, where DL is 1.05 ug/g. After each aqueous extraction, the water-soil mixtures were centrifuged to remove fine soil particles and decanted into a 250-ml separatory funnel. The water was extracted sequentially with three 80-ml portions of methylene chloride. The methylene chloride extracts were passed through sodium sulfate, collected in a K-D apparatus, and concentrated to 5 ml. The extract was cleaned by passage through a Florisil® Sep-Pak® and analyzed by HPLC with UV detection at 395 nm.

These recoveries, presented in Table 3-4, were good. After completion of initial tests on natural soil, which showed similar recoveries, full documentation of the method was attempted.

As observed during the 35DNP method development task, low recoveries were obtained when the 35DNA extracts were allowed to stand in methylene chloride overnight prior to K-D concentration. Again, this problem was circumvented by performing the extraction and concentration steps in 1 day and by using acid-washed glassware.

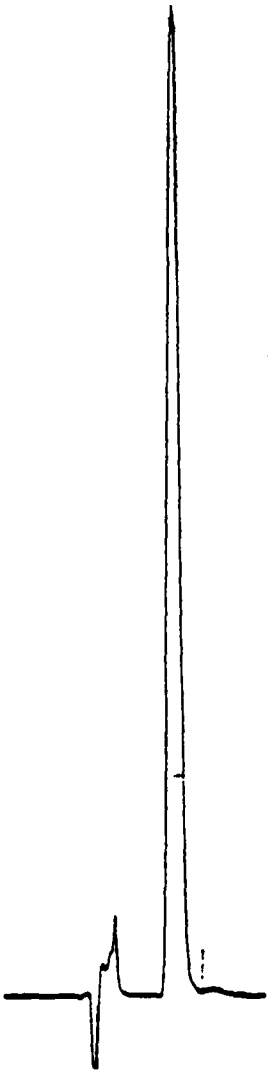
The USATHAMA August 1980 QA Plan requires that samples be spiked with spiking solutions prepared in the extracting solvent. Enough solvent to completely cover the soil sample is added. The sample is then allowed to sit for several hours to air-dry. Because water was the extracting solvent in this procedure and would not quickly evaporate, it was felt that the recovery results could be biased toward higher recoveries. To test whether the presence of excess water biased the recovery results, a set of natural soil samples was spiked using a methylene chloride spiking solvent and thoroughly air-dried for several hours to ensure that the 35DNA was on the soil and not dissolved in the spiking solvent. This set of samples was then extracted with water and analyzed by the proposed method. The recoveries obtained were slightly lower but within the 95-percent confidence range for recoveries from natural soil determined during the documentation of the method. A chromatogram of 35DNA in natural soil spiked at 2.1 ug/g is presented in Figure 3-6.

Method Documentation--The analytes were spiked into standard and natural soils at 0, 0.5 DL, 1 DL, 2 DL, 5 DL, and 10 DL, where DL is 1.1 ug/g. These spiked samples were analyzed with the entire procedure. Replicates were analyzed at the six spiking levels on each of 4 separate days. The detection limit was calculated from found-versus-target concentration data using the Hubaux and Vos (1970) procedure. The precision and accuracy of the method were calculated from the slope and standard error of the estimate ($S_{y.x}$) of the least-squares

Table 3-4. Recovery of 35DNA from Standard Soil Carried through the Soil Method (Water Spiking Solvent)

Concentration Spiked (ug/g)	Concentration Found (ug/g)	% Recovery
0.52	0.36	69
1.05	0.88	84
2.10	1.67	79
5.25	4.69	89
10.5	9.26	88

Source: ESE, 1982.



Column: Ultrasphere-ODS (4.6-mm ID x 25 cm, 5 um)
Detector: Perkin-Elmer LC-75 variable-wavelength detector (λ = 395 nm)
Flow Rate: 1 ml/min
Mobile Phase: 70% methanol/30% water
Injection Volume: 250 ul, fixed-loop

SOURCE: ESE, 1982

Figure 3-6

CHROMATOGRAM OF 35DNA IN NATURAL SOIL SPIKED AT 2.1 ug/g

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regression line of the found-versus-target concentration data. The detection limit of the method was 1.1 ug/g in natural soil and 0.53 ug/g in standard soil. The accuracy of the method for 35DNA over the spiking range (0.53 to 10.5 ug/g) was 92 percent for the natural soil. The average percent imprecision over the spiking range for natural soil was 14 percent. The documented analytical method and data for 35DNA are presented in Appendix C.

3.3 2,4,6-TRINITROBENZALDEHYDE (ATNBA)

3.3.1 Physical and Chemical Properties

ATNBA is found in trinitrotoluene manufacturing wastewaters and is a photodegradation product of TNT (Burlinson, Kaplan, and Adams, 1973). Some of the physical properties and alternate nomenclature for ATNBA are listed in Table 3-5. The chemical structure of this compound is presented in Figure 3-1.

ATNBA is explosive and is decomposed by UV light to the dibasic acid, 2,2'-dicarboxy-3,3',5,5'-tetranitroazoxybenzene (Marendic and Norris, 1973). Its solutions must be protected from exposure to UV light by use of amber glass or wrapping of solution containers.

In basic solution, ATNBA hydrolyzes rapidly to 135TNB. Aqueous potassium hydroxide or sodium carbonate, for example, participate in ATNBA hydrolysis to TNB and the alkali formate salt (Little, 1980). In aqueous solutions at a pH of 7, ATNBA undergoes hydrolysis to TNB at a much slower rate.

ATNBA is known to be unstable at high temperatures and decomposes rapidly at 200°C (Little, 1980). It also can be degraded to TNB in solution by dissolved oxygen. ATNBA is slightly soluble in water but very soluble in organic solvents such as alcohol, ether, acetone, benzene, and chloroform.

Table 3-5. Alternate Nomenclature and Physical and Chemical Properties of ATNBA

A. ALTERNATE NOMENCLATURE AND CAS REGISTRY NUMBER

Analyte	Alternate Nomenclature	CAS Registry Number
ATNBA	2,4,6-Trinitrobenzene carbonal 2,4,6-Trinitrobenzene carboxaldehyde	606-34-8

B. PHYSICAL AND CHEMICAL PROPERTIES

Analyte	Formula	Melting Point (°C)	Boiling Point (°C)	Density (g/ml)
ATNBA	$C_7H_3O_7N_3$	119	--	--

Source: ESE, 1982.

A UV spectrum of the ATNBA SARM obtained from USATHAMA dissolved in methanol solvent was obtained (Figure 3-7) and is in agreement with the Sadtler Index spectra (Sadtler Research Laboratories, Inc., 1970). The maximum occurs at 215 nm, and the absorbance is 2.5 times weaker at 254 nm.

3.3.2 Results of Literature Search for Analytical Methods

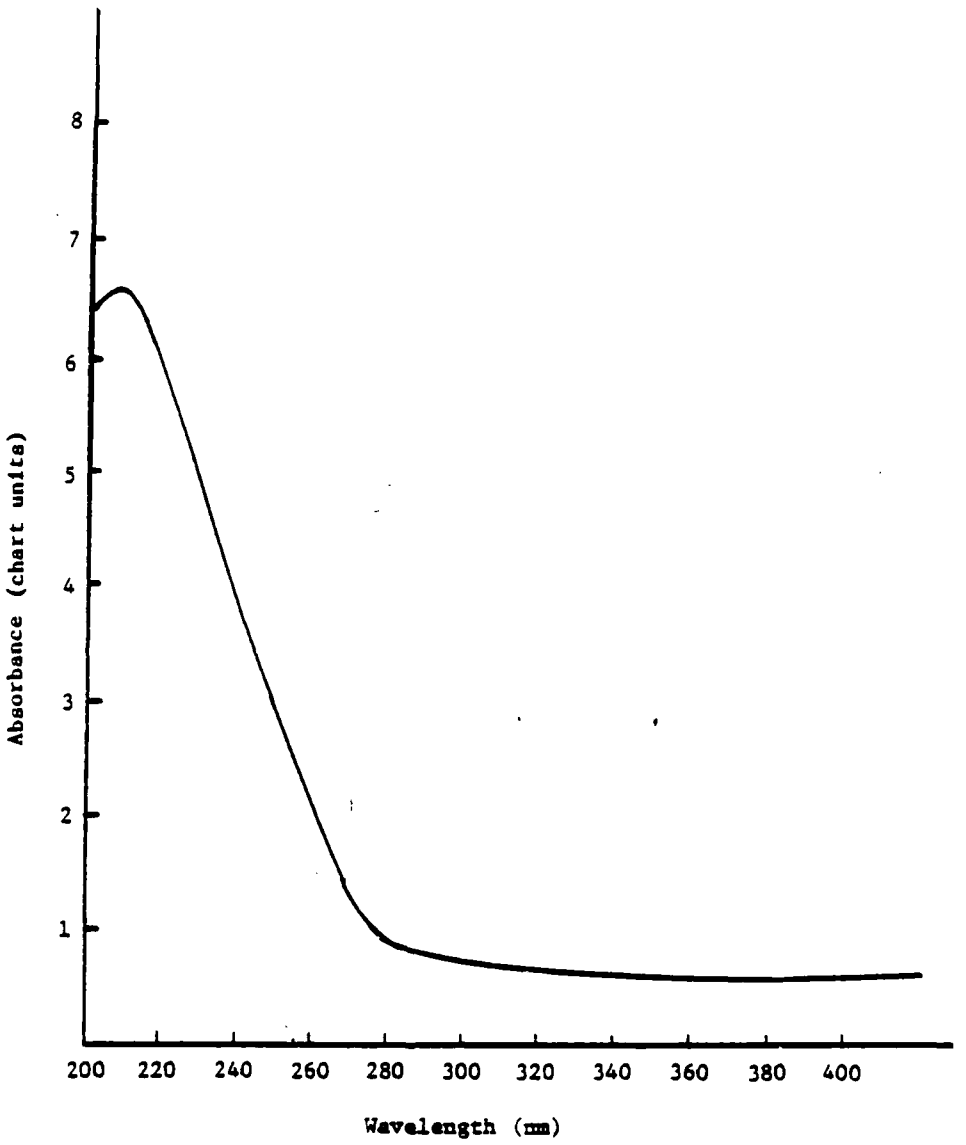
Few analytical methods were found for the analysis of ATNBA in water or soil. It was apparent that analytical research in this area had not been pursued, probably due to the unstable nature of this compound. A reference to the use of thin-layer chromatography (TLC) was found in which TLC was used to quantify ATNBA isolated as one of the photo-decomposition products of TNT (Burlinson, Kaplan, and Adams, 1973).

3.3.3 Preliminary Experiments and Method Optimization--Water

The possibility of using HPLC or GC methods of analysis was considered as these methods are generally straightforward, selective, and sensitive. HPLC was especially favored due to the previous TLC work on ATNBA analysis. The potential problem of the thermal decomposition of ATNBA at high temperatures effectively ruled out GC as the technique of choice. HPLC was selected as the preferred approach.

After settling on an HPLC approach, experimental efforts were directed towards finding the optimum liquid chromatographic detector and column conditions. An electrochemical detector (Bioanalytical Systems, LC-4, glassy-carbon electrode) was investigated for applicability to ATNBA analysis due to the presence of the oxidizable aldehyde moiety. In the oxidative mode with a potential of 1.15 volts, ATNBA response was weak, and oxidative electrochemical detection did not appear feasible.

Efforts then were directed toward the use of a UV detector for ATNBA. Although the maximum UV absorbance of ATNBA occurs near 215 nm, the use of this wavelength as the analytical monitoring wavelength was not especially favored due to the nonspecificity of this wavelength and the



SOURCE: ESE, 1982

Figure 3-7

UV ABSORPTION SPECTRUM OF ATNBA IN METHANOL

Prepared for:

U.S. Army Toxic and Hazardous Materials Agency

Aberdeen Proving Ground, Maryland

potential for interferences from many organic species which absorb strongly in this region of the UV spectrum. The absorbance at 254 nm, although approximately 40% less intense, was sufficiently sensitive to allow the detection of 10 ng of ATNBA using a 100-ul injection volume. The use of this wavelength also was favored because it would provide more selectivity and is a standard wavelength in fixed-wavelength UV detectors.

Initial chromatographic conditions for ATNBA analysis were established on a 5-um Ultrasphere-ODS column (2.5 cm by 4.5-mm ID) using a mobile phase of 60% methanol/40% phosphate buffer, adjusted to a pH of 7. Due to the hydrolytic degradation of ATNBA observed in methanol water solutions, a switch to acetonitrile/water mobile phase was made during the early method optimization experiments. However, experiments revealed that the use of methanol/water mobile phase had little effect on the chromatographic stability of ATNBA, and, in fact, provided better separation of ATNBA from potentially interfering nitroaromatic compounds. A final mobile phase composition of 55% methanol/45% water was selected as the optimum chromatographic choice. The retention times of other possible nitroaromatic interferences under these final ATNBA chromatographic conditions are listed in Table 3-6. No peak overlap and interference problems were observed. A chromatogram of a natural water extract spiked at 2.4 ug/L with ATNBA is shown in Figure 3-8.

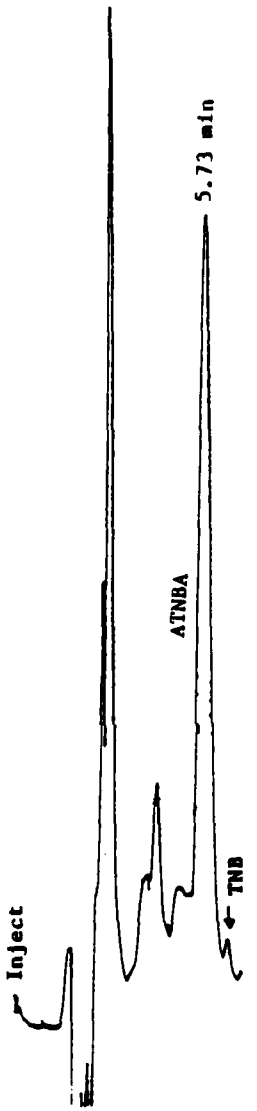
The development of an extraction procedure for ATNBA in water was complicated by its tendency to undergo hydrolysis in aqueous solutions. This property was intensified at elevated temperatures, a fact which ruled out the use of K-D concentration in polar solvents. This hydrolytic behavior of ATNBA was first observed when a standard solution (19.6 ug/L) made up in 60% methanol/40% water, pH = 7 phosphate buffer turned bright pink after standing for several hours at room temperature. The formation of the pink color was accompanied by the appearance of a new unknown peak in the HPLC chromatogram of this standard solution and a decrease in the size of the ATNBA peak. The loss of ATNBA and the

Table 3-6. Retention Times of Selected Nitroaromatic Compounds Under HPLC Conditions for ATNBA Analysis

Compound	Retention Time* (minutes)
ATNBA	5.8
135TNB	6.7
Tetryl	8.6
Nitrobenzene (NB)	8.7
246TNT	9.7
35DNP	11.7
RDX	4.9
HMX	3.6
24 DNT	11.6
26 DNT	11.3
13 DNB	7.9

* HPLC conditions: Ultrasphere-ODS column, 5 um, 25 cm by 4.5-mm ID; flow rate--1 ml/min; 55% methanol/45% water.

Source: ESE, 1982.



Column: Ultrasphere-ODS (4.6-mm ID x 25 cm, 5 um)
Detector: Altex Model 153 fixed-wavelength detector
(λ = 254 nm)
Flow Rate: 1 ml/min
Mobile Phase: 55% methanol/45% water
Injection Volume: 250 ul, fixed-loop

SOURCE: ESE, 1982.

Figure 3-8

CHROMATOGRAM OF ATNBA IN NATURAL WATER SPIKED AT 2.36 ug/L

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formation of the new peak occurred rather rapidly, showing an approximately 20% decrease in ATNBA concentration per hour. Making the standard solution basic by the addition of sodium hydroxide caused an almost instantaneous loss of ATNBA and complete conversion to the unknown peak. The rate of this conversion could be slowed considerably by the adjustment of the solution pH to 3 or less.

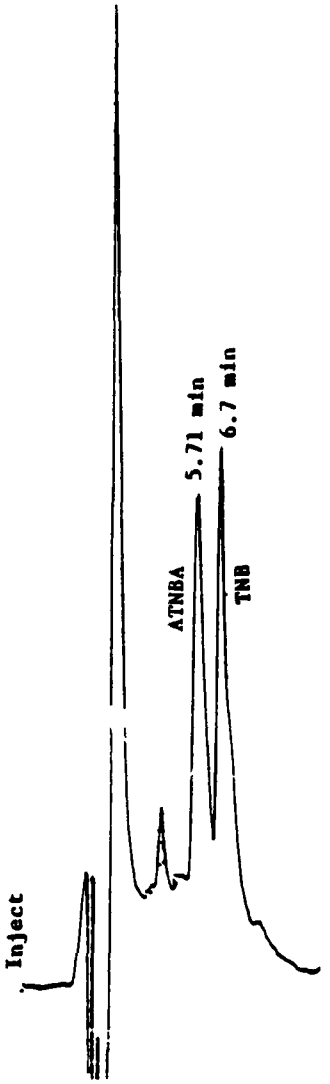
During later experiments involving evaporation of methylene chloride extracts and solvent-exchange to methanol at elevated temperatures (approximately 85° to 100°C), the same pink coloration was observed. Analysis of the methanol concentrates revealed that almost 100% loss of ATNBA had occurred with complete conversion to an unknown peak with the same retention time as that noted in the earlier experiments. Little (1980) states that in basic solution, ATNBA is hydrolyzed to TNB and formate ion. The retention time of the unknown peak appearing in the chromatograms of the methanol concentrates was identical to that of TNB run under the same chromatographic conditions. This tentative identification of the unknown peak as TNB was verified by extracting the methanolic concentrate containing the 100% hydrolyzed ATNBA with toluene and analyzing this extract by GC using a GC/EC procedure developed for nitroaromatics. The GC retention times for the hydrolysis product and TNB were identical. These results combined with the earlier experiments confirmed that ATNBA undergoes rapid hydrolysis in aqueous solutions of methanol at room temperature and in methanol at elevated temperatures.

Further investigations involving extract K-D concentration at elevated temperature (85° to 100°C) using acetonitrile as the final exchange solvent instead of methanol showed that decomposition occurred in acetonitrile also, but to a lesser extent. Because little decomposition was observed in methylene chloride extracts, the hydrolytic behavior of ATNBA in acetonitrile and methanol appeared to be related to the polarity of the solvents, the use of elevated evaporation temperatures, and possibly the presence of trace water in these polar solvents. Because of these stability problems, K-D evaporation techniques were not

employed for ATNBA analyses. The use of rotary evaporation under reduced pressure and temperatures no greater than 35°C was evaluated as an alternative concentration technique. Initial spiking experiments into methylene chloride with solvent-exchange to acetonitrile using this technique were successful in providing good recoveries with little decomposition.

The hydrolysis of ATNBA in aqueous solutions raised concerns about the proper solvent to use for preparation of stock and working standard solutions and the stability of the sample extracts. As mentioned previously, ATNBA was hydrolyzed rapidly (approximately 20 percent per hour) in methanol/water solutions. On the other hand, ATNBA solutions in 50% acetonitrile/50% water exhibited a slower hydrolytic rate for ATNBA. A chromatogram of a 5-mg/L standard solution in 50% acetonitrile/50% water which had been left at room temperature overnight is presented in Figure 3-9. The hydrolysis of ATNBA is substantial as evidenced by the large TNB peak in this chromatogram. A 5-mg/L standard in 50% acetonitrile/50% water protected from exposure to light by foil wrapping, showed a 9-percent-per-hour rate of decrease in ATNBA concentration. More dilute standards (0.03 mg/L) exhibited a higher rate of decrease of approximately 13 percent per hour. This rate could be slowed considerably by storage of the solutions at 4°C.

The stability of stock solutions of approximately 1,000-mg/L concentration was compared using acetonitrile and methylene chloride solvents. ATNBA stock solutions made up in acetonitrile (dried with sodium sulfate), protected from light, and stored at -15°C in a freezer showed little degradation (less than 4% in 7 days) even though a light pink coloration still developed. If oxygen was excluded from the flask, storage time could be increased to several weeks with little degradation observed. In methylene chloride solvent, ATNBA stock solutions exhibited even more stable behavior than in acetonitrile, but this solvent was not favored due to chromatography problems caused by



Concentration of TNBA: 5 mg/L
Column: Ultrasphere-ODS (4.6-mm ID x 25 cm, 5 um)
Detector: Altex Model 153 fixed-wavelength detector
(λ = 254 nm)
Flow Rate: 1 ml/min
Mobile Phase: 55% methanol/45% water
Injection Volume: 250 ul, fixed-loop

SOURCE: ESE, 1982.

Figure 3-9

CHROMATOGRAM OF PARTIALLY HYDROLYZED ATNBA STANDARD SOLUTION IN 50% ACETONITRILE / 50% WATER

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Aberdeen Proving Ground, Maryland

residual methylene chloride in the working standard solutions prepared from the methylene chloride stock.

The results of these stability experiments dictated that degassed, dried acetonitrile be used as the solvent for stock ATNBA solutions and that these solutions should be stored under nitrogen at -15°C. Working stock solutions were made up fresh daily from the stocks and diluted into 50% acetonitrile/50% water. Sample extracts were kept in acetonitrile and stored at -15°C until just before analysis, when an appropriate volume of degassed water was added to dilute the extracts to 50% acetonitrile/50% water solution.

The effect of direct sunlight on ATNBA stability was investigated briefly. According to Little (1980), the photodecomposition product of ATNBA is the dibasic acid, 2,2'-dicarboxy-3,3',5,5'-tetranitroazoxybenzene. This compound is ionic at a pH of 7 and would tend to elute on the solvent front under the chromatographic conditions used for ATNBA analysis. A 0.5-mg/L ATNBA standard solution in 50% acetonitrile/50% water solvent in a Pyrex® flask was left in direct sunlight for 4 hours. After exposure, the standard was analyzed. ATNBA had decomposed completely, but only 3% of it had hydrolyzed to TNB. A large peak appeared in the chromatogram at the solvent front, indicating the possible formation of the suspected photolysis product. The results of this experiment, although not conclusive, strongly indicated that in direct sunlight, photolysis is the dominant process in predominantly aqueous solutions as compared to hydrolysis at a pH of 7.

Development of a cleanup procedure for ATNBA extracts involved the evaluation of a silica-gel fractionation scheme similar to one used in the RDX, HMX, and PETN multiple-analyte method (Section 4.1). ATNBA (9.6 ug) was spiked into hexane and added to a 60/200-mesh (Davidson, Grade 950) silica-gel column (20 cm by 10.5-mm ID). The column was eluted with a sequence of solvents of varying polarity, as shown in Table 3-7. Each fraction was collected and analyzed for ATNBA. Any

Table 3-7. Silica-Gel Sep-Pak® Fractionation Scheme and Recovery of ATNBA*

Fraction	Solvent Composition	Elution Volume (ml)	% Recovery		
			ATNBA	ATNBA as TNB	Total
1	Hexane	25	--†	--	0
2	20% methylene chloride/ 80% hexane	10	--	--	0
3	50% methylene chloride/ 50% hexane	10	--	--	0
4	100% methylene chloride	10	5.1	1.5	6.6
5	5% acetonitrile/95% methylene chloride	10	9.1	3.3	12.4
6	20% acetonitrile/80% methylene chloride	10	74.7	5.9	80.6
7	50% acetonitrile/50% methylene chloride	10	--	--	0
Total Recovered					99.6

* 9.6 ug of ATNBA spiked onto column.
† Not analyzed.

Source: ESE, 1982.

decomposition of ATNBA to TNB was also noted, and the recoveries of ATNBA were adjusted for the proportionate amount of TNB formed. Significant decomposition of ATNBA occurred in these tests because these preliminary experiments included a heated K-D solvent evaporation step and were performed before the change to rotovap evaporation was instituted. The recoveries indicated that complete elution of ATNBA was possible with 20% acetonitrile/80% methylene chloride and that selective cleanup of nonpolar compounds could be achieved without loss of ATNBA by pre-elution of the column with 50% methylene chloride/50% hexane.

Because of their convenience and reproducibility, both silica gel and Florisil® Sep-Pak® cartridges obtained from Waters Associates, Milford, Massachusetts, were evaluated for the cleanup procedure. The results of these experiments are presented in Tables 3-8 and 3-9. Florisil® was investigated due to the good recoveries obtained during work-up of the 35DNP and 35DNA methods. Both silica-gel and Florisil® Sep-Pak® separations provided good recoveries for ATNBA. The data indicated, as before on silica-gel columns, that 50% hexane/50% methylene chloride or 50% pentane/50% methylene chloride did not elute ATNBA and thus could be used as a preliminary elution solvent to eliminate interferences. Pentane was favored over hexane in the mixed solvent due to its lower boiling point and ease of removal by rotovap evaporation. Florisil® in Sep-Pak® cartridges was eventually chosen as the optimum cleanup adsorbent because it provided recoveries as well as silica gel and had been successfully employed for other analytes.

Method Documentation--Quantitative method documentation was performed in accordance with the project QC Plan. The documented method and data are presented in Appendix C. ATNBA was spiked into standard water and natural water at 0, 0.5 DL, 1 DL, 2 DL, 5 DL, and 10 DL, where DL is 1.18 ug/L. These spiked samples were analyzed with the entire procedure, including the Florisil® cleanup step. Replicates were analyzed at the six spiking levels on each of 4 separate days. The detection limit was calculated from the found-versus-target concentrationn data using

Table 3-8. Silica-Gel Sep-Pak® Fractionation Scheme and Recovery of ATNBA*

Fraction	E°†	Solvent Composition	Elution Volume (ml)	% Recovery
1	0.27	50% hexane/50% methylene chloride	10	0
2	0.33	100% methylene chloride	5	25
3	0.65	25% methanol/75% methylene chloride	2	75
				100

* 101 ug of ATNBA spiked onto cartridge.
† Solvent strength parameter on silica gel.

Source: ESE, 1982.

Table 3-9. Florisil® Sep-Pak® Fractionation Scheme and Recovery of ATNBA*

Fraction	E°†	Solvent Composition	Elution Volume (ml)	% Recovery
1	0.27	50% pentane/50% methylene chloride	10	0
2	0.37	100% methylene chloride	5	37
3	0.43	20% acetonitrile/80% methylene chloride	5	48
4	0.50	100% acetonitrile	5	0.5
				85.5

* 50 ug of ATNBA spiked onto cartridge.
† Solvent strength parameters on silica gel are similar to those on Florisil®.

Source: ESE, 1982.

the Hubaux and Vos (1970) procedure. The precision and accuracy of the method in natural and standard water were calculated from the standard error of the estimate and slope, respectively, of the least-squares regression line of the found-versus-target concentration data. The detection limit of the method was 3.3 ug/L in standard water and 2.1 ug/L in natural water. The accuracy of the method over the spiking range (0.59 to 11.8 ug/L) was 87 percent for natural water. The average percent imprecision for natural water was 18 percent.

Care was taken during documentation to ensure the stability of standards and sample extracts. Sample extracts were left in acetonitrile solvent before analysis and made up in 50% acetonitrile/50% water immediately before analysis. All sample extracts and standard solutions were protected from light by wrapping the containers with aluminum foil.

3.3.4 Preliminary Experiments and Method Optimization--Soil

The method developed for ATNBA in water was adapted for ATNBA in soils. The water method consisted of methylene chloride extraction, concentration by rotary evaporation, Florisil® Sep-Pak® cleanup, and reverse-phase HPLC/UV analysis. The ease of decomposition of ATNBA due to hydrolysis had been noted during methods development for the water method, and procedures to minimize hydrolysis had been implemented.

ATNBA has a relatively high water solubility, estimated at 2 to 10 mg/L (Little, 1980. Therefore, the ATNBA-in-soil method, which involved water extraction of the soil and analysis of the extract using the developed water method, was thought to be an optimum procedure for evaluation. This procedure also was favored because of the low octanol/water partition coefficient (K_{ow}). This coefficient is approximately 2 for ATNBA, which implied there was little tendency to find this compound adsorbed in sediments. Several natural and standard soil samples (20 grams) were spiked with 845 ug of ATNBA and extracted by shaking with three consecutive 35-ml aliquots of water. The extraction efficiency was monitored by direct injection of the aqueous

extracts on the HPLC column. Greater than 90% extraction of ATNBA from the natural soil and greater than 80% extraction from the standard soil were achieved in the first 35-ml aliquot. Complete recovery of ATNBA was attainable by three 35-ml extractions.

Although good recoveries were obtained with the water extraction procedure, ATNBA was known to have a greater solubility in methylene chloride. Also, an extraction of ATNBA from soil by methylene chloride would eliminate the back-extraction step of the water extract with methylene chloride. The procedure was, therefore, modified to use a methylene chloride extraction of the soil rather than a water extraction. Use of methylene chloride extraction was also preferable due to the tendency of ATNBA to hydrolyze in aqueous media.

A tentative extraction and analysis procedure was decided upon at this time. The procedure consisted of placing 20 grams of soil into a 50-ml Teflon®-lined, screw-capped, glass centrifuge tube. The soil is extracted by shaking vigorously for several minutes with three successive 35-ml portions of methylene chloride. The tube was vented several times during the extraction procedure to release methylene chloride pressure. After each shaking, the sample was centrifuged at 3,000 rpm to settle the soil. The methylene chloride layer was decanted off into a 250-ml round-bottom flask. Each of the methylene chloride extracts was combined together in the round-bottom flask, which was subsequently attached to a rotovap for evaporation of the methylene chloride. The evaporation was conducted at 35°C, and the residue was redissolved in 50% pentane/50% methylene chloride. After Florisil® Sep-Pak® cleanup, the extract was analyzed by the developed HPLC method for water. Details of this procedure are given in the documented method in Appendix A. This tentative method was tested on a partial documentation set in natural soil. The samples were spiked in the range of 0.5 ug/g to 10.8 ug/g ATNBA. Recoveries are listed in Table 3-10. These results were excellent, and method documentation proceeded with no

Table 3-10. Recoveries of ATNBA Extracted from Natural Soil

Concentration Spiked (ug/g)	Concentration Found (ug/g)	% Recovery
0	0	--
0.51	0.49	95.1
2.04	1.96	96.1
10.2	9.47	92.8

Source: ESE, 1982.

further changes. A chromatogram of ATNBA in natural soil spiked at 2.04 ug/g is presented in Figure 3-10.

In natural samples containing significant amounts of moisture, the same procedure would be applicable, except that the methylene chloride layers are removed by pipet rather than by decantation during the same extraction to avoid the introduction of water into the methylene chloride extracts.

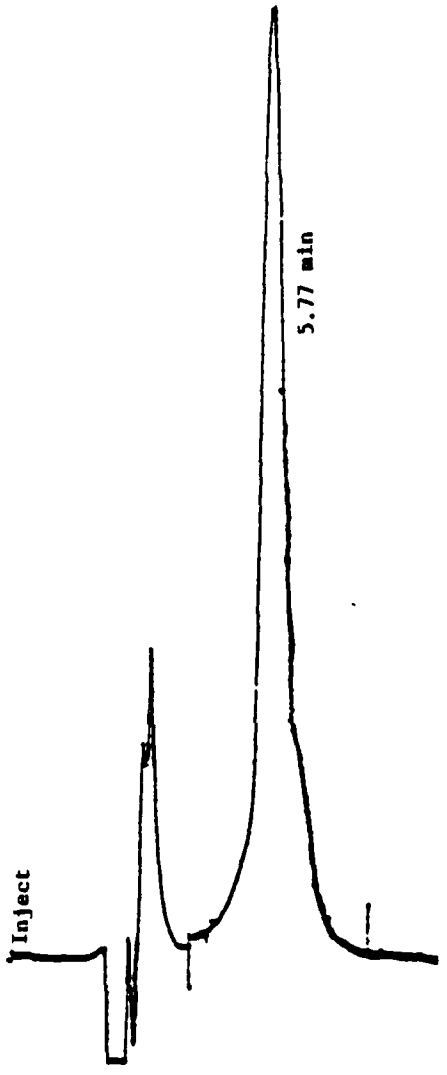
Method Documentation--Quantitative method documentation was performed by spiking ATNBA into standard and natural soils at 0, 0.5 DL, 1 DL, 2 DL, 5 DL, and 10 DL, where DL is 1.02 ug/g. The spiked samples were analyzed using the full analytical procedure, including the Florisil® cleanup step. The documented method and data are presented in Appendix C.

Four sets of spiked samples were analyzed, one on each of 4 separate days, for each soil type. The detection limit of the method was calculated from the found-versus-target concentration data using the method of Hubaux and Vos (1970). Precision and accuracy data were calculated from the standard error of the estimate (Sy.x) and slope of the least-squares regression line of the found-versus-target concentration data. The detection limit of the method is 2.0 ug/g in standard soil and 3.5 ug/g in natural soil. The accuracy of the method over the spiking range (0.51 to 10.2 ug/g) was 87 percent for natural soil. The average percent imprecision was 22 percent for natural soil.

3.4 TDGCL

3.4.1 Physical and Chemical Properties

Some of the physical properties and alternate nomenclature of TDGCL are listed in Table 3-11. The chemical structure of TDGCL is presented in Figure 3-11. TDGCL has a relatively high boiling point of 165°C, as do most glycols. It is also highly soluble in water, alcohols, and chlorinated and aromatic solvents. TDGCL is an aqueous



Column: Ultrasphere-ODS (4.6-mm ID x 25 cm, 5 um)
Detector: Altex Model 153 fixed-wavelength detector
(λ = 254 nm)
Flow Rate: 1 ml/min
Mobile Phase: 55% methanol/45% water
Injection Volume: 250 ul, fixed-loop

SOURCE: ESE, 1982.

Figure 3-10

CHROMATOGRAM OF ATNBA IN NATURAL SOIL SPIKED AT 2.04 ug/g

Prepared for:
U.S. Army Toxic and Hazardous Materials Agency
Aberdeen Proving Ground, Maryland

Table 3-11. Alternate Nomenclature and Physical and Chemical Properties of TDGCL

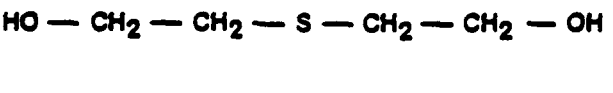
A. ALTERNATE NOMENCLATURE AND CAS REGISTRY NUMBER

Analyte	Alternate Nomenclature	CAS Registry Number
TDGCL	2,2'-Thiodiethanol Bis (B-hydroxyethyl) Sulfide	111-48-8

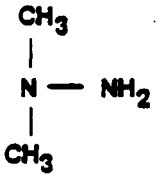
B. PHYSICAL AND CHEMICAL PROPERTIES

Analyte	Formula	Melting Point (°C)	Boiling Point (°C)	Density (g/ml)
TDGCL	$C_4H_{10}O_2S$	-10	165	1.1819

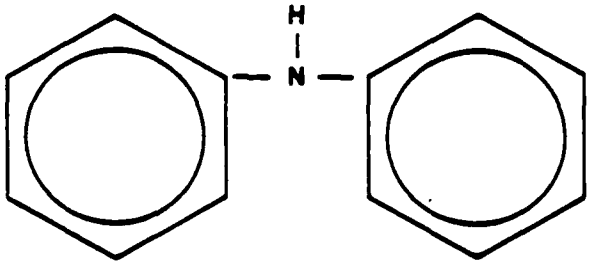
Source: ESE, 1982.



TDGCL



UDMH



DPA

SOURCE: ESE, 1982.

Figure 3-11

CHEMICAL STRUCTURES OF TDGCL, UDMH, AND DPA

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U.S. Army Toxic and Hazardous Materials Agency

Aberdeen Proving Ground, Maryland

hydrolysis product of mustard gas (i.e., dichlorodiethylsulfide) which is relatively stable in aqueous solution.

3.4.2 Results of Literature Search for Analytical Methods

During the computerized literature search for analytical methods for TDGCL, no specific methods for TDGCL were found. However, some methods for glycols in general were reported. Typically, glycols have been analyzed by GC using flame ionization detection (Yamanis et al., 1975). DiCorcia and Sangeri (1979) showed that the use of tetrahydroxyethylene-diamine (THEED) as a tailing reducer and selectivity modifier on an inert adsorbing medium such as Carbopack-C gave untailed peaks even for a few nanograms of diols and glycerol. A spectrophotometric determination of mono-, di-, and triethylene glycols in surface waters, based on the oxidation of the glycols to aldehydes, was reported by Evans and Dennis (1973).

3.4.3 Preliminary Experiments and Method Optimization--Water

GC methods were initially investigated for the analysis of TDGCL because they have been used successfully in the past for glycol analyses. An attempt to obtain acceptable GC conditions and suitable extraction solvents was undertaken. Initial experiments with standard solutions using a flame ionization detector showed that there were problems with using methanol and ethyl acetate as standard solution solvents, due to considerable chromatographic interferences and excessive peak tailing. Methylene chloride seemed to give acceptable results and, therefore, was chosen for the preparation of TDGCL standard solutions. However, workable analysis conditions still had to be determined.

Further experiments indicated that the best column packing material was a 10% SP-1000 on 80/100-mesh Supelcoport. An acceptable peak shape for TDGCL was obtained on this column. Other GC columns were subsequently investigated, but severe tailing or poor response precluded their use. A change to use of a flame photometric detector (FPD) in the sulfur mode was made to obtain enhanced selectivity. However, GC analysis, even

using an FPD in the sulfur mode, did not give the sensitivity required; thus, TDGCL analysis was attempted by HPLC, and experiments to determine suitable HPLC conditions were initiated.

Optimum HPLC conditions were established on an Ultrasphere-ODS (4.6-mm ID by 25 cm) column (5-um particle size) using a mobile phase of 0.05 M potassium phosphate buffer aqueous solution and a flow rate of 1 ml/minute. No organic solvent was used in the mobile phase. Detection was by a Perkin-Elmer LC-75 variable-wavelength detector at 215 nm, using the end absorption band of TDGCL. The injection volume was 250 ul using a fixed-loop injector. Acceptable peak shapes and sensitivity were obtained. Under these conditions 1 ug of TDGCL could be detected. During the method documentation experiments, the HPLC retention time for TDGCL was noticed to vary with the age and condition of the column. This variability was reduced to a great extent by the use of the phosphate buffer in the mobile phase, but a definite shift to longer retention times was noted during the natural sample documentation. This behavior required frequent standardization of the column to assure correct peak identification.

Experiments showed that TDGCL could not be extracted from water by methylene chloride. This fact was used to advantage later in the development of the water method because extraction of water with methylene chloride could be used as a pre-extraction cleanup step. Methylene chloride would remove organic interferences while leaving TDGCL in the water phase. The selected extraction approach consisted of an adjustment of the aqueous sample pH to 3, followed by methylene chloride extraction. The methylene chloride extract, which contained any extractable acidic/neutral organic compounds (which could be possible interferences) was discarded, but not TDGCL.

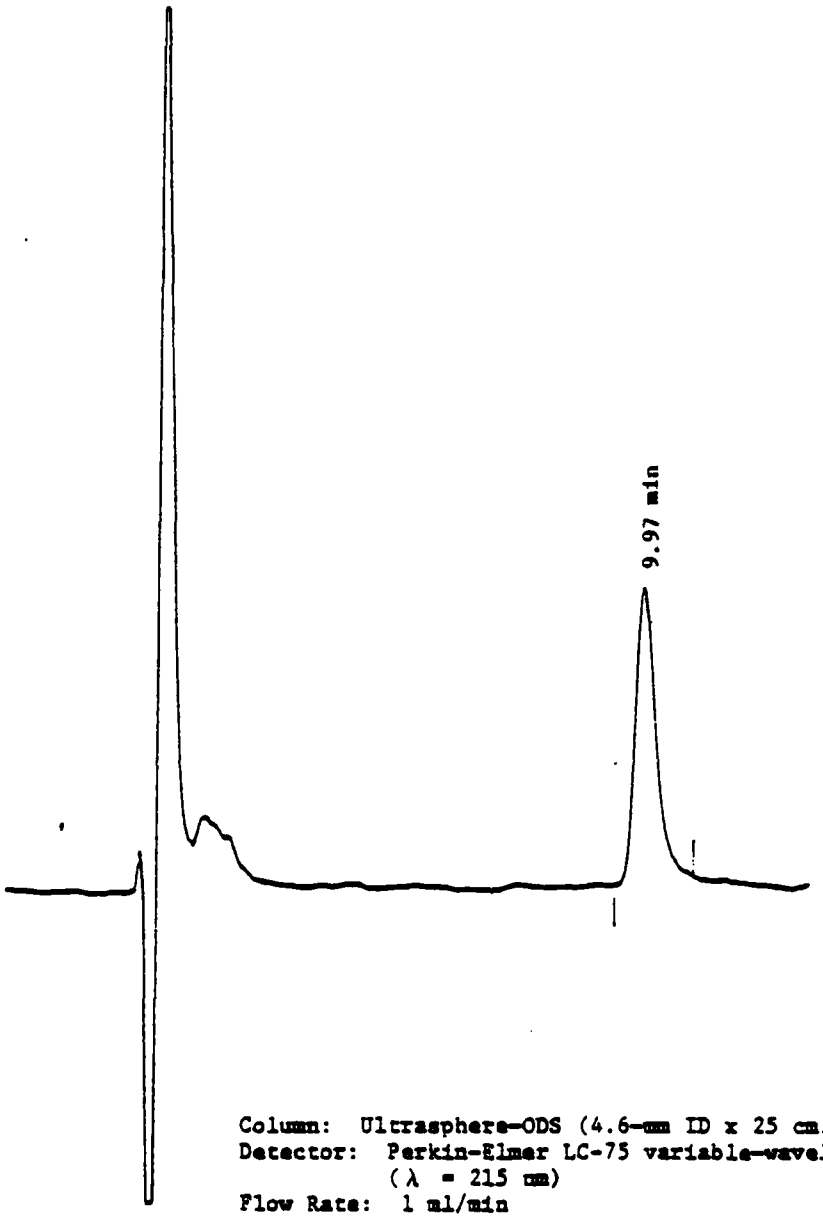
After the preliminary methylene chloride extraction, the water sample is neutralized to a pH of 7 and reduced in volume to 5 ml by boiling on a hot plate. This means of concentrating TDGCL takes advantage of its

high boiling point (165°C) and extreme miscibility in water. The TDGCL concentrate was then injected into the LC.

The concentration achievable by the boildown step was limited to a 100-to-1 factor because evaporation of a 500-ml sample volume below 5 ml caused serious chromatographic problems such as peak shape distortion, tubing blockage, and baseline disturbances due to the high concentration of salts in the final sample. Major problems with the method as delineated above were not encountered. Therefore, full documentation of the method in standard water commenced. A detection limit of 20 ug/L was achievable in standard water using a final sample volume of 5 ml and an initial sample volume of 500 ml. A chromatogram of TDGCL spiked into standard water is presented in Figure 3-12.

Application of the method to natural water samples resulted in considerable chromatographic interferences in the form of numerous extraneous interfering peaks in the chromatograms and high baseline backgrounds. Poor recoveries for spiked samples were also obtained. The boildown time used to concentrate the aqueous phase appeared to contribute to the low recoveries. The time required for sample boildown was therefore reduced by performing the boildown in large, 1-liter beakers rather than in 1-liter Erlenmeyer flasks as originally performed. The time required for boildown was reduced to approximately one-half the time previously required, or approximately 2.5 hours.

It was observed that the relative amount of chromatographic interferences in natural water was increased by the boildown procedure in comparison with the amount of interferences in the natural water before boildown probably due to the decomposition of large naturally occurring humic and fulvic acids. Further cleanup was apparently needed for natural water. Column cleanup using XAD-7 resin was tried and found to be effective in significantly reducing interferences. Recoveries near 100% of TDGCL spiked at the 1-mg/L level were obtained in aqueous samples which were passed through the resin column. This cleanup step



Column: Ultrasphere-ODS (4.6-mm ID x 25 cm, 5 um)
Detector: Perkin-Elmer LC-75 variable-wavelength detector
(λ = 215 nm)
Flow Rate: 1 ml/min
Mobile Phase: 100% 0.05M potassium-dihydrogen-phosphate buffer, pH = 6
Injection Volume: 250 ul, fixed-loop

SOURCE: ESE, 1982.

Figure 3-12

CHROMATOGRAM OF TDGCL IN STANDARD WATER SPIKED AT 37 ug/L

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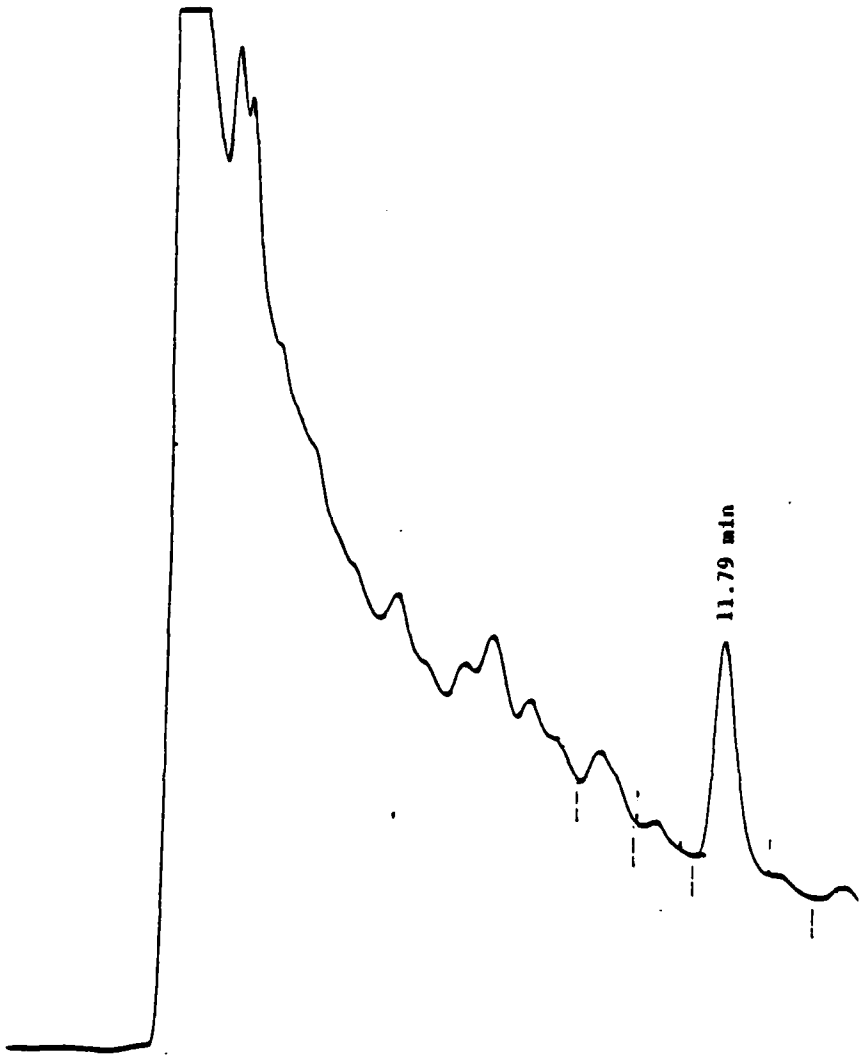
was added to the standard water method for TDGCL analysis. The resin is cleaned before use by vigorously shaking it for 20 minutes on a wrist-action shaker in a flask which contains methanol. This process is repeated three times. The resin is then thoroughly rinsed with organic-free water. The resin could be regenerated a maximum of three times between samples before the buildup of contaminants became excessive. Although interferences were substantially reduced by the column cleanup procedure, the background level in this natural water still restricted the detection limit of the method to approximately 120 ug/L.

A chromatogram of TDGCL in natural water spiked at 400 ug/L is presented in Figure 3-13.

Method Documentation--Quantitative method documentation was performed in accordance with the project QC Plan. TDGCL was spiked into standard and natural water at 0, 0.5 DL, 1 DL, 2 DL, 5 DL, and 10 DL, where DL is 18.6 ug/L in standard water and 80 ug/L in natural water. These spiked samples were analyzed with the entire procedure. Replicates were analyzed at the six spiking levels on each of 4 separate days. The detection limit was calculated from the found-versus-target concentration data using the method of Hubaux and Vos (1970). The detection limits achieved were 27 ug/L in standard water and 119 ug/L in natural water.

The precision and accuracy of the method were calculated from the standard error of the estimate ($S_{y.x}$) and slope, respectively, of the least-squares regression line of the found-versus-target concentration data. The accuracy of the method over the spiking range of 40 to 800 ug/L in natural water was 65 percent. The average percent imprecision of the method in natural water was 14 percent.



Column: Ultrasphere-ODS (4.6-mm ID x 25 cm, 5 um)
Detector: Perkin-Elmer LC-75 variable-wavelength detector
(λ = 215 nm)
Flow Rate: 1 ml/min
Mobile Phase: 100% 0.05M potassium-dihydrogen-phosphate buffer, pH = 6
Injection Volume: 250 ul, fixed-loop

SOURCE: ESE, 1982.

Figure 3-13

CHROMATOGRAM OF TDGCL IN NATURAL WATER SPIKED AT 400 ug/L

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U.S. Army Toxic and Hazardous Materials Agency
Aberdeen Proving Ground, Maryland

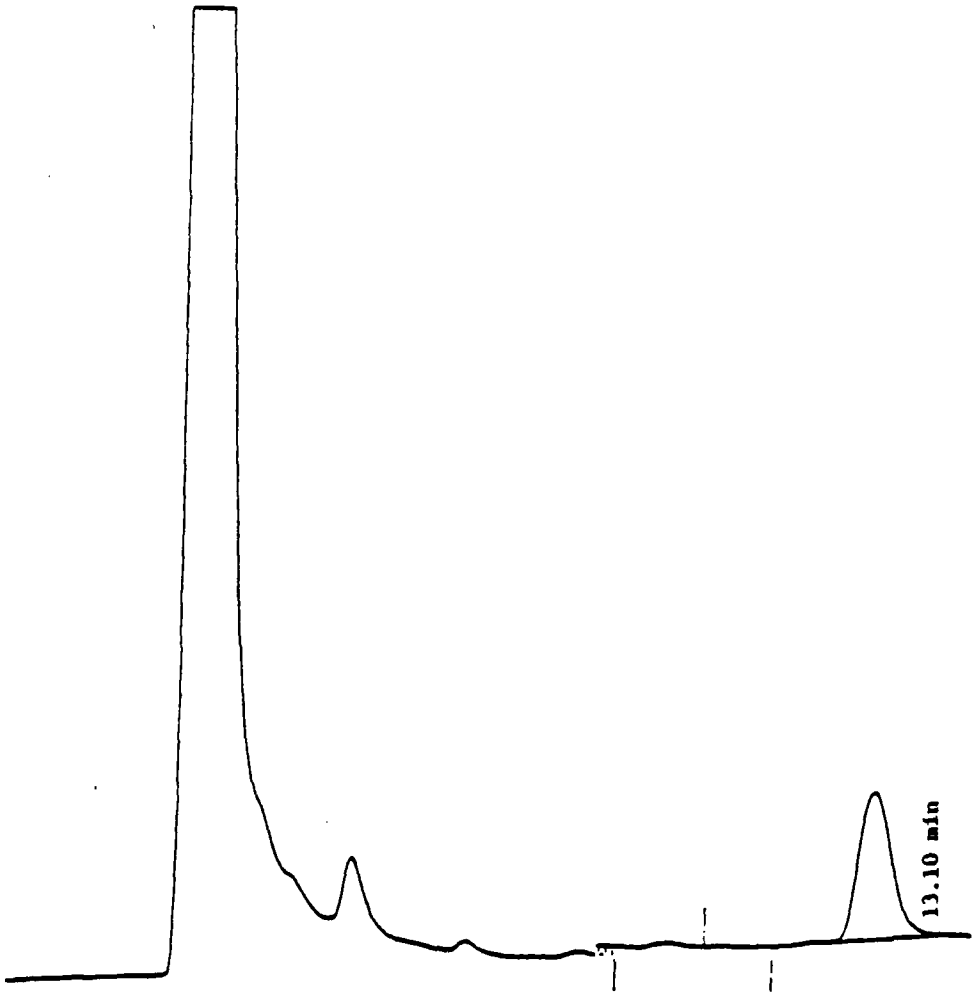
3.4.4 Preliminary Experiments and Method Optimization--Soil

The natural water method for the analysis of TDGCL was modified for application to soil matrices. Because of the polarity of the glycols, it was not possible to extract them from soil quantitatively by organic solvent extraction. Because of this fact, and its high solubility in water, TDGCL was first extracted from standard soil by water. This was performed by shaking the soil-water mixture for 15 minutes on a wrist-action shaker. The sample was then filtered through a Whatman No. 1 filter, passed through the XAD-7 resin for cleanup, concentrated by boiling, and analyzed by HPLC.

No major interference problems were encountered in applying this method to standard soil, but emulsion formation and extremely slow filtration were encountered in natural soil samples. Centrifugation of the water-soil mixture in a glass centrifuge tube was substituted for the filtration step in the procedure resulting in greatly improved recoveries, faster analysis times, and lessened emulsion formation.

A chromatogram of TDGCL in natural soil spiked at 4 ug/g is presented in Figure 3-14.

Method Documentation--Quantitative method documentation was performed in accordance with the project QC Plan. TDGCL was spiked into standard and natural soil at 0, 0.5 DL, 1 DL, 2 DL, 5 DL, and 10 DL, where DL is 2.0 ug/g. These spiked samples were analyzed with the entire procedure. Replicates were analyzed at the six spiking levels on each of 4 separate days. The detection limit was calculated from the found-versus-target concentration daca using the method of Hubaux and Vos (1970). Precision and accuracy data were calculated from the standard error of the estimate and slope of the least-squares regression line of the found-versus-target concentration data. The documented method and data results are presented in Appendix C. The detection limit in standard soil was 4.1 ug/g and 2.2 ug/g in natural soil. The accuracy of the method in the natural soil over the spiking range of 1.0 to 20 ug/g was



Column: Ultrasphere-ODS (4.6-mm ID x 25 cm, 5 um)
Detector: Perkin-Elmer LC-75 variable-wavelength detector
(λ = 21[illegible] nm)
Flow Rate: 1 ml/min
Mobile Phase: 100% 0.05M potassium-dihydrogen-phosphate buffer, pH = 6
Injection Volume: 250 uL, fixed-loop

SOURCE: ESE, 1982.

Figure 3-14

CHROMATOGRAM OF TDGCL IN NATURAL SOIL SPIKED AT 4 ug/g

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U.S. Army Toxic and Hazardous Materials Agency
Aberdeen Proving Ground, Maryland

96 percent. The average percent imprecision over the spiking range was 9 percent for natural soil.

3.5 UNSYMMETRICAL DIMETHYL HYDRAZINE (UDMH)

3.5.1 Physical and Chemical Properties

UDMH is a powerful reducing agent used as a base in rocket fuel formulations. Some of the physical properties of UDMH are listed in Table 3-12. The chemical structure of UDMH is presented in Figure 3-11. It is highly corrosive and irritating to the skin, eyes, and mucous membranes. UDMH is very soluble in water and ethanol, and practically insoluble in ether.

UDMH is readily oxidized in alkaline solution by a number of oxidants (HgO, halogens, and halates) to produce tetrazine. Tetrazines are inherently instable and split out N_2 under thermal or protolytic conditions. In acidic solutions, UDMH reacts to form diazonium salts, $(CH_3)_2N^+ = NH\ X^-$, which react as dienophiles with conjugated dienes in the Diels-Alder reaction. Hydrazines reduce many commonly found metal ions to lower valence states or to the metals themselves. More than 23 metal ions have been shown to react with hydrazines.

UDMH undergoes hydrolysis in aqueous solutions. The half-life for this process has been found to be less than 1 day. The kinetics of the decomposition of UDMH in aqueous solutions were briefly examined in this study, and the results are reported in Section 3.5.2.

3.5.2 Results of Literature Search for Analytical Methods

A computerized literature search for analytical methods for the determination of UDMH was conducted. Methods found involved titrimetry, GC, and liquid chromatography. Malone and Anderson (1969) analyzed mixtures of hydrazine and 1,1-dimethylhydrazine by a titrimetric method. The total hydrazine content is found by titration with iodate and addition of salicylaldehyde converts the hydrazine to salicylaldazine

Table 3-12. Alternate Nomenclature and Physical and Chemical Properties of UDMH

A. ALTERNATE NOMENCLATURE AND CAS REGISTRY NUMBER

Analyte	Alternate Nomenclature	CAS Registry Number
UDMH	Asym-dimethylhydrazine 1,1-Dimethylhydrazine Unsym-dimethylhydrazine N,N-Dimethylhydrazine Dimazine	57-14-7

B. PHYSICAL AND CHEMICAL PROPERTIES

Analyte	Molecular Formula	Melting Point	Boiling Point	Density (g/ml)
UDMH	$C_2H_8N_2$	-58°C	63.9°C	0.791

Source: ESE, 1982.

(disalicylhydrazine), which is removed by filtration. Subsequent titration with standard potassium iodate gives the UDMH or hydrazine content.

Selim and Warner (1978) prepared a derivative of hydrazine in water by reaction with acetone to form the acetone azine. This derivative was determined by GC using a nitrogen/phosphorus detector. The detection limit reported for this procedure was 0.1 ug/L. Derivation was also used by Newsome (1980), who reacted UDMH with pentafluorobenzoyl chloride and analyzed the resulting derivative by GC with EC detection. GC with a nitrogen-specific detector was used by Timbrell et al. (1977) to analyze the hydrazine derivative formed by reaction of hydrazines and p-chlorobenzaldehyde. Raulin et al. (1980) used GC with flame ionization detection for the analysis of volatile amines and showed that the limit of detectability of these organic compounds can be lower than 0.1 ng. Dee and Webb (1967) demonstrated that through proper choice of GC column packing materials, separation of water, UDMH, monomethyl hydrazine (MMH), and hydrazine can be completed in all proportions with little or no adsorption and with symmetrical peaks. The use of a GC concentrator to improve analysis of low levels of airborne hydrazine and UDMH was reported by Mazur et al. (1980).

Discussions so far have emphasized GC methods for UDMH analysis. However, in recent years, liquid chromatography has also been used. Abdou et al. (1977) reported on a rapid, sensitive liquid chromatographic method for the determination of hydrazine and 1,1-dimethylhydrazine, separately or in mixtures of varying proportions. The method involves salicylaldehyde derivative formation followed by liquid chromatographic determination on a reversed-phase (octadecylsilane) column. Different HPLC columns for the separation of 1,2-dimethylhydrazine metabolites were investigated by Fiala et al. (1976). The columns used were C_{18}/Corasil, Bondapak-C_{18}, and Aminex A-27.

3.5.3 Preliminary Experiments and Method Optimization--Water

Based on articles in the literature, a GC approach to UDMH analysis in water was attempted. Newsome (1980) reported the analysis of dimethylhydrazines using derivatization with pentafluorobenzoyl chloride (PFBC). Analysis of the PFBC derivative was by GC with EC detection. The use of this derivatization technique was evaluated for the analysis of UDMH at the 1- to 15 µg/L level in standard water. The procedure consisted of the reaction of PFBC with UDMH in highly alkaline (pH>12) solutions, extraction of the derivative with methylene chloride, evaporation of the extract to dryness, and redissolution in toluene or hexane. Chromatography of the derivative was performed on a 6-foot, 1% SP-1000 column with a column temperature of 150°C. These experiments did not yield consistent results because the extent of formation of the derivative was highly variable. Attempts to control the experimental conditions and to assure the presence of fresh UDMH stock solutions and fresh reagents did not improve the reliability of the method.

The approach of Selim and Warner (1978), who prepared a derivative of hydrazine in water by reaction with acetone to form the acetone azine, was evaluated next. The derivative (1,1-dimethylacetone hydrazone) was prepared by reaction of UDMH with excess acetone. The reaction product was distilled, and the fraction boiling at approximately 90°C was collected. This colorless liquid was dissolved in toluene at the mg/L level and injected on various GC columns at several temperatures. The derivative proved unstable on all the columns tried and under various chromatographic conditions. The columns tested included:

1. 1.5% OV-17/1.95% QF1 on 100/120-mesh Gas Chrom Q,
2. 0.1% SP 1000 on 80/100-mesh Carbopak C, and
3. 10% SP 2340 on 80/100-mesh Supelcoport.

Another approach involved derivatization of UDMH with benzaldehyde. Abdou, Medwick, and Bailey (1977) developed an HPLC method for the analysis of hydrazine and UDMH involving derivatization with salicylaldehyde. This method was applicable to the hydrazine compounds in

organic media (isopropanol) and not to aqueous media. Monomethyl-hydrazine formed an unstable derivative with salicylaldehyde. ESE attempted to make the benzaldehyde derivative of UDMH. The reaction occurred readily in toluene; however, hydrolysis appeared to be a strong competing reaction in water. Attempts to extract UDMH from water followed by reaction to form the derivative were unsuccessful due to the high water solubility of UDMH. Toluene, hexane, and ethyl acetate were tried as extraction agents. Salting-out from a highly basic (pH >12) water solution did not noticeably increase the extraction efficiency. To avoid the relatively reactive aldehyde proton of benzaldehyde and thus minimize hydrolysis, acetophenone was tried as the derivatization agent, but there was no noticeable improvement in the stability of this ketone derivative over the aldehyde derivative.

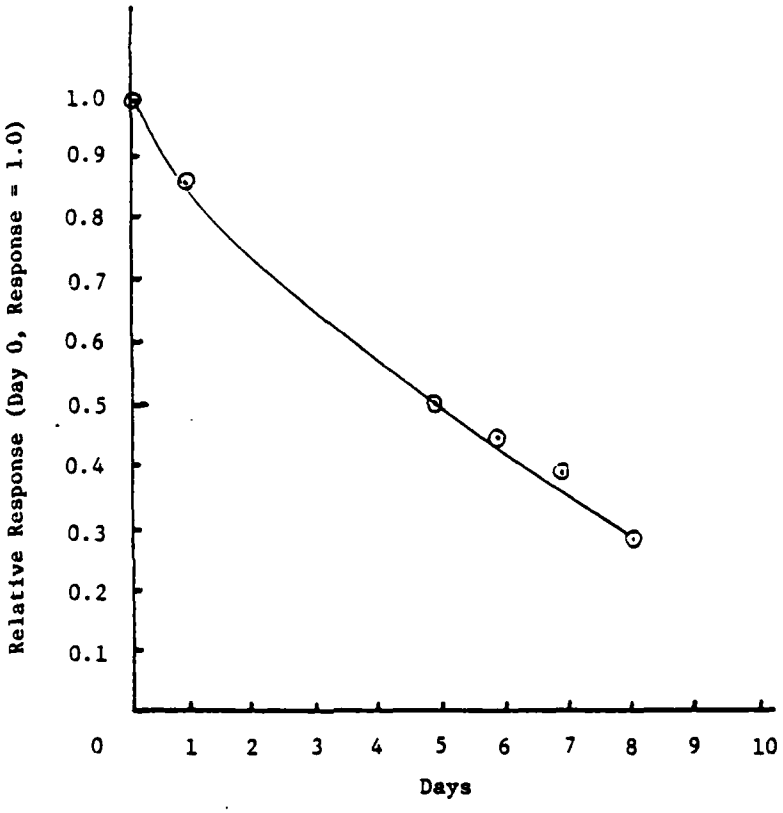
Early methods development work on the possible use of HPLC for the analysis of UDMH concentrated on establishing the optimum column and detector conditions. Using a UV detector at 210 nm for detection, it was observed that UDMH could be chromatographed on a Zorbax-C8 column (25 cm by 4.6-mm ID) using a 55% methanol/45% water mobile phase. Because UDMH has a weak end absorption bond at wavelengths less than 210 nm, the sensitivity of UV detection was very low.

The UDMH chemical structure possessed a free amine moiety and because of its strong reducing agent properties, oxidative electrochemical detection of UDMH was investigated. Using a glassy-carbon electrode and an electrode potential of +1.0 volt, a large response was obtained for UDMH. The mobile phase in this case was modified by the use of a 0.09 M potassium dihydrogen phosphate buffer at a pH of 7 instead of pure water mixed with methanol to provide a conducting solvent for proper operation of the electrochemical detector. Based on these results, direct aqueous injection HPLC with electrochemical detection was selected as the optimum method for further development. A flow rate of 1 ml/min and an injection volume of 250 ul were used in the analysis.

Using freshly prepared standards of UDMH in the HPLC mobile phase, loss of UDMH response was observed to occur with time of standing for the standards. The decrease in peak response with time after preparation of a 50-ug/L UDMH standard is illustrated in Figure 3-15. A 70-percent loss in UDMH concentration was observed over a 9-day period. [In this test, the instrumental response for an internal standard (phenol) was used to assure constant instrument sensitivity.] This solution was not protected from light. All stock and standard solutions were wrapped in aluminum foil and, though the rate of loss was reduced, substantial loss of UDMH was observed. A further precaution was taken by using amber bottles with no headspace to eliminate contact with air and thus minimize oxidation of UDMH.

By taking the precautions previously described and using freshly prepared (daily) standard solutions and spiked samples, documentation of the method for UDMH in standard water was conducted. The method consisted of the direct aqueous injection of the aqueous sample after 50% dilution with acetonitrile. The dilution with acetonitrile was necessary to keep the chromatographic mobile phase and sample solvent the same and minimize peak shape distortion and baseline disturbances in the chromatogram. Sample cleanup was not necessary owing to the specificity of the electrochemical detector. However, this method may be subject to interferences from compounds which can be readily oxidized under an electrochemical potential of +0.9 volt. Phenolic compounds are included in this class. Chromatographic conditions were selected to minimize interferences from the commonly found priority pollutant phenols.

Method documentation included spiking samples at 0, 0.5 DL, 1 DL, 2 DL, 5 DL, and 10 DL, where DL was 10.4 ug/L. The samples were separately spiked and analyzed on 4 consecutive days. The stock and standard solutions were prepared fresh daily. Documentation of the method was also performed in natural water with a DL level of 10.8 ug/L. Because the UDMH standards decomposed rapidly, new stocks had to be prepared



Concentration: UDMH, 50 ug/L
Solvent: 50% acetonitrile/50% 0.09M PO_4^{-3} buffer, pH = 7
Column: Zorbax-C8 (25 cm x 4.6-mm ID)
Detector: Bioanalytical LC-4, +0.9v vs. S.C.E., glassy-carbon electrode

SOURCE: ESE, 1982.

Figure 3-15

DECREASE IN INSTRUMENTAL RESPONSE OF 50-ug/L UDMH STANDARD

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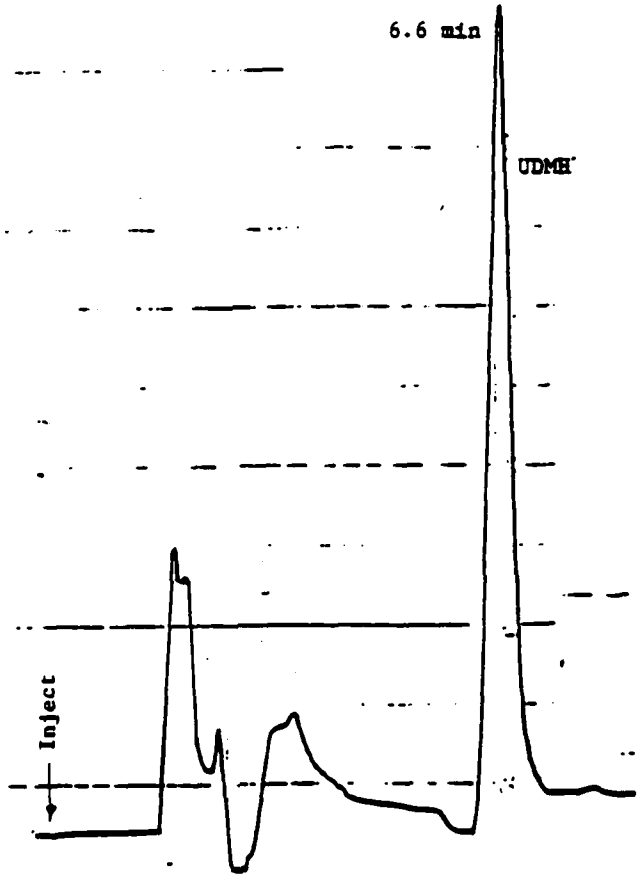
daily for the spiking experiments. Exact duplication of the spiking concentration levels was not feasible. The target concentrations were very closely spaced about the average concentration for that level with a standard deviation of no more than 2 percent. The detection limit of the method was calculated by applying the method of Hubaux and Vos (1970) to the found-versus-target concentration data. The detection limits of the method in standard and natural water were 16 and 11 ug/L, respectively. The accuracy of the method was equivalent to the slope of the least-squares regression line of the found-versus-target concentration data. The precision was calculated from the standard error of the estimate ($S_{y.x}$) of this line. The accuracy of the method in natural water over the spiking range (5.1 to 108 ug/L) is 120 percent. There appeared to be a positive bias in the method which appeared to be due to the response of the electrochemical detector. The average percent imprecision over the spiking range was 14 percent.

A chromatogram of a natural water sample spiked at 54 ug/L with UDMH is presented in Figure 3-16. The fully documented method and data are presented in Appendix C.

3.5.4 Aqueous Phase Kinetic Studies

Preliminary observations regarding the loss of UDMH in standard solutions had been made at the onset of this methods development task. More detailed data on this phenomenon were desired, and several kinetic studies were conducted.

In particular, studies on the effect of acidic, neutral, and basic sample pH on the loss of UDMH in solution were performed. Stability experiments were performed at pHs 5, 7, and 9 in standard water at a UDMH concentration of 270 ug/L. This concentration was selected to ensure adequate signal response on the detector by allowing at least a tenfold range for the response to decrease and still be detectable. Three amber, 60-ml, septum-sealed bottles were filled with freshly



Column: Zorbax-C8 (25 cm x 4.6-mm ID)
Mobile Phase: 50% acetonitrile/50% 0.09M phosphate buffer, pH = 7
Detector: BAS LC-4, electrochemical, +0.9v vs. S.C.E., Att x 20-nA
Injection Volume: 250 ul

SOURCE: ESE, 1982.

Figure 3-16
CHROMATOGRAM OF UDMH IN NATURAL WATER SPIKED AT 54 ug/L

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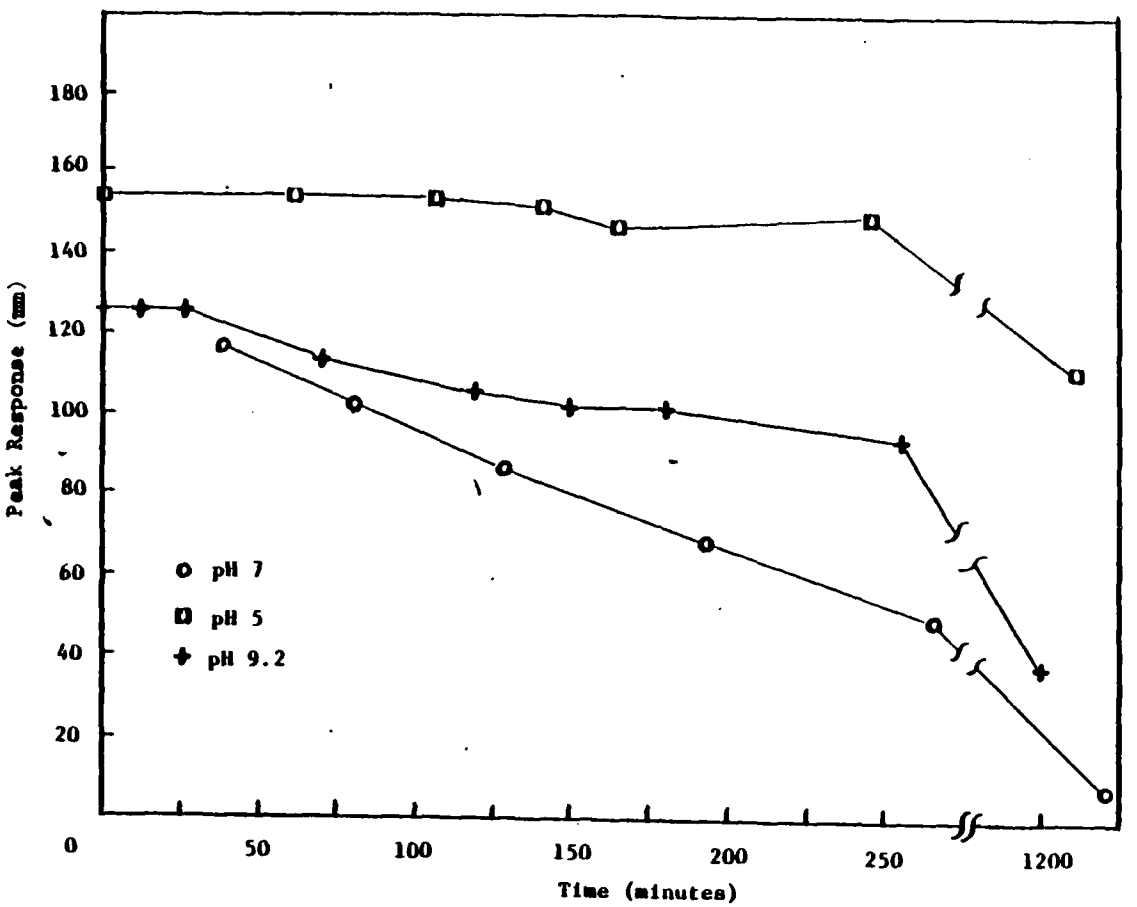
prepared UDMH solutions prepared in the appropriate pH buffer. For a pH of 7, a mixed potassium-dihydrogen-phosphate/potassium-monohydrogen-phosphate buffer was used (4.22 grams of KH_2PO_4 and 3.31 grams of K_2HPO_4 in 1 liter of water). The pH-5 buffer was prepared by dissolving 6.67 grams of KH_2PO_4 and 0.135 gram of K_2HPO_4 in 1 liter of water. A sodium borate buffer (0.01 M) was prepared for the pH = 9 test. Three samples were prepared at each pH at the same concentration of UDMH. The solutions were sealed in the amber bottles with no headspace to minimize volatilization of UDMH. The amber bottles contained a Teflon®-lined septum. Immediately before sealing, each sample was analyzed by HPLC and the instrumental response to the UDMH peak was noted. Each bottle was subsequently analyzed at regular time intervals for more than 20 hours. The samples were thermostatted at 30°C for the duration of the experiment.

The electrochemical detector sensitivity, which can change due to changes in the electrode surfaces over a period of several days, was monitored on a regular basis throughout the stability tests by measuring the instrument response to a phenol standard. Instrument drift would be noted in any increased or decreased response to phenol.

The results of these experiments are presented in Figure 3-17. The data indicate that the fastest rate of decrease in UDMH concentration with time is observed in the pH-7 solution, which shows a 41% decrease in concentration over a 4.4-hour period. After approximately 20 hours, the concentration had dropped to 8.1% of the original concentration. The rate of decrease is less at pH 9.2, with the least decrease observed at pH 5. A decrease of only 27% is observed during a 20-hour period at this pH.

Based on these results, a more thorough experiment using a larger number of equally spaced data points was conducted at a pH of 7. Assuming that the decomposition or loss of UDMH was a first-order kinetic process, the resultant data were plotted in the following form:



SOURCE: ESE, 1982.

Figure 3-17

DEGRADATION OF 270 ug/L OF UDMH AT 30°C IN STANDARD WATER AT pHs 5, 7, AND 9.2

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$$\ln\left[\frac{a_o}{a_o - c}\right] = kt$$

where a_o = initial concentration at t = 0,

c = concentration loss of UDMH in time t,

k = first-order rate constant, and

t = time after initiation in seconds.

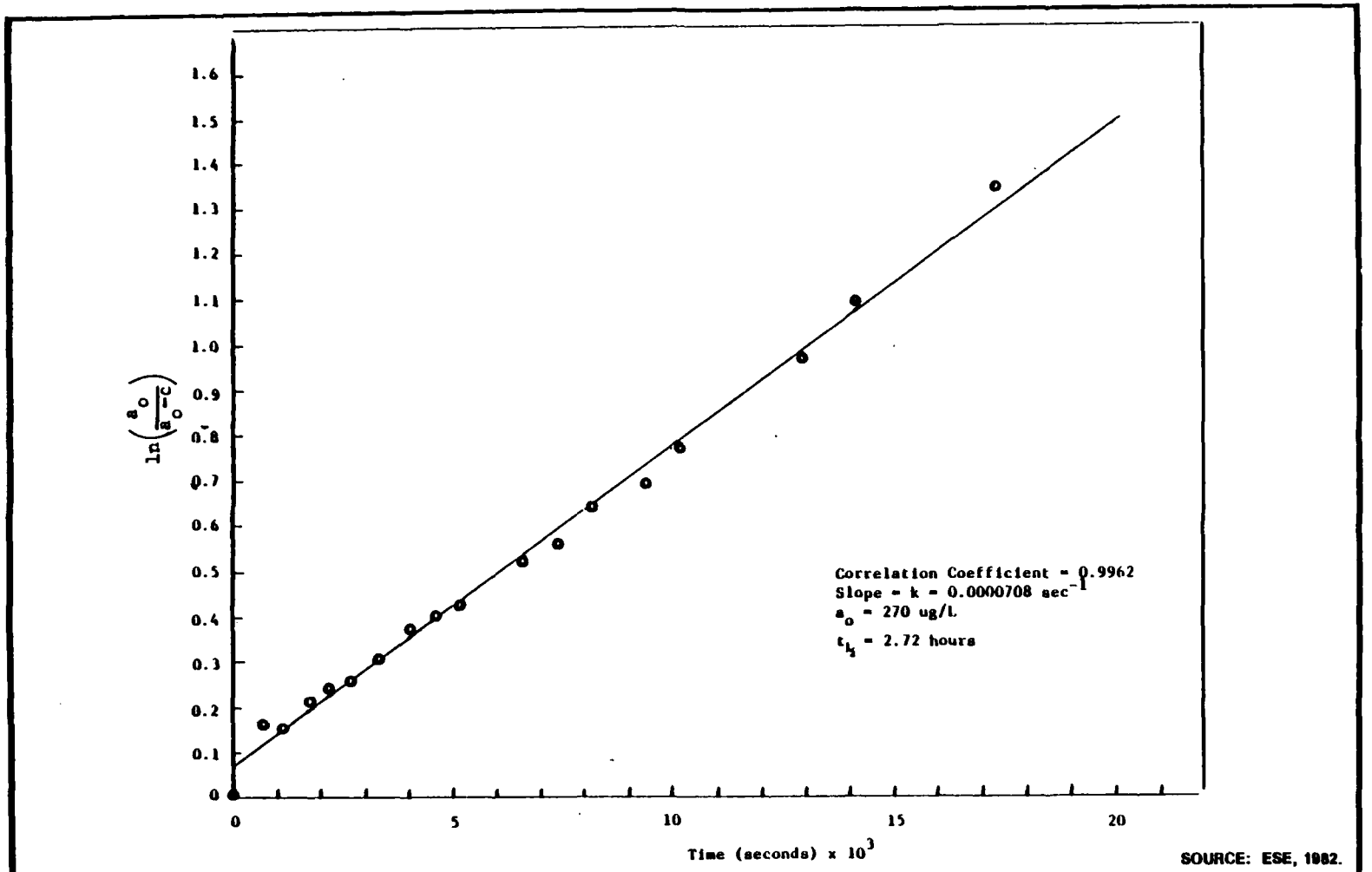
These data are presented in Table 3-13 and are plotted in Figure 3-18. The plotted data have a good linear correlation coefficient of 0.9962 for the least-squares regression line of the data, indicating good agreement with the assumption of first-order degradation kinetics. The first-order rate constant calculated from these data is 7.08×10^{-5} sec^{-1}, and the half-life, $t_{1/2}$, is 2.72 hours. The half-life is the time required for a species to decay to one-half of its original concentration. Simultaneous with the loss in UDMH concentration, an increase in response for an unidentified peak in the sample chromatogram was observed. The peak eluted earlier than UDMH under the chromatographic conditions employed. A series of chromatograms showing this unknown peak at various times during the kinetic experiments is shown in Figure 3-19. Attempts to identify this peak were beyond the scope of this project.

To investigate the effect of increased temperature on UDMH degradatic , a set of kinetic experiments was performed at 50°C and a pH of 7. The concentrations of UDMH and other conditions (except temperature) were identical to the described experiments at 30°C. The results of the first-order plot are presented in Figure 3-20. Also shown are the data from an identical experiment using vacuum-degassed buffer solution to evaluate the effect of oxygen on the degradation. All these experiments were conducted in buffer solutions which had been filtered through a 0.22-um filter to remove microorganisms and using sterilized glassware.

Table 3-13. Variation of UDMH Aqueous Concentration with Time at pH 7 and 30°C

t Time (sec) x 10^3	$(a_o - c)$ Concentration (ug/L)	$\frac{a_o}{(a_o - c)}$	$\ln\left\|\frac{a_o}{(a_o - c)}\right\|$
0	270(a_o)	1	0
0.546	229	1.179	0.1647
1.062	231	1.169	0.1560
1.650	217	1.244	0.2185
2.172	212	1.274	0.2418
2.700	208	1.291	0.2609
3.300	199	1.357	0.3052
4.068	186	1.452	0.3727
4.644	181	1.492	0.3999
5.232	177	1.525	0.4220
6.600	160	1.6875	0.5232
7.440	154	1.7532	0.5615
8.220	142	1.9014	0.6426
9.420	135	2.0000	0.6931
10.200	125	2.1600	0.7701
13.020	102	2.6471	0.9734
14.220	90.5	2.9834	1.0931
17.340	70.7	3.8190	1.3400
20.520	53.5	5.0467	1.6187

Source: ESE, 1982.



SOURCE: ESE, 1982.

Figure 3-18

KINETICS OF UDMH DEGRADATION IN WATER AT pH 7 AND 30°C

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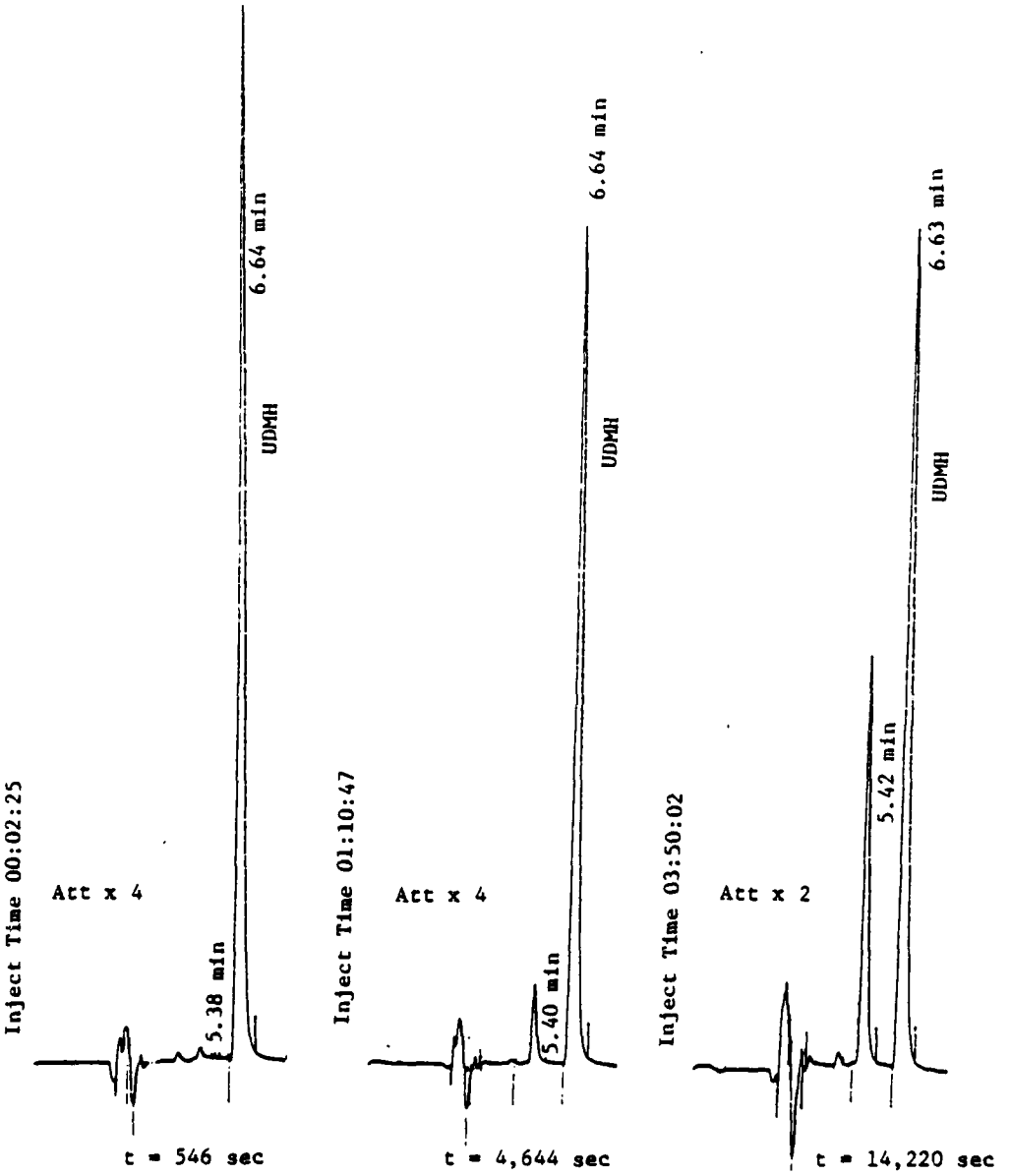

SOURCE: ESE, 1982.

Figure 3-19

CHROMATOGRAMS OF UDMH SOLUTIONS DURING pH-7 KINETIC STUDIES

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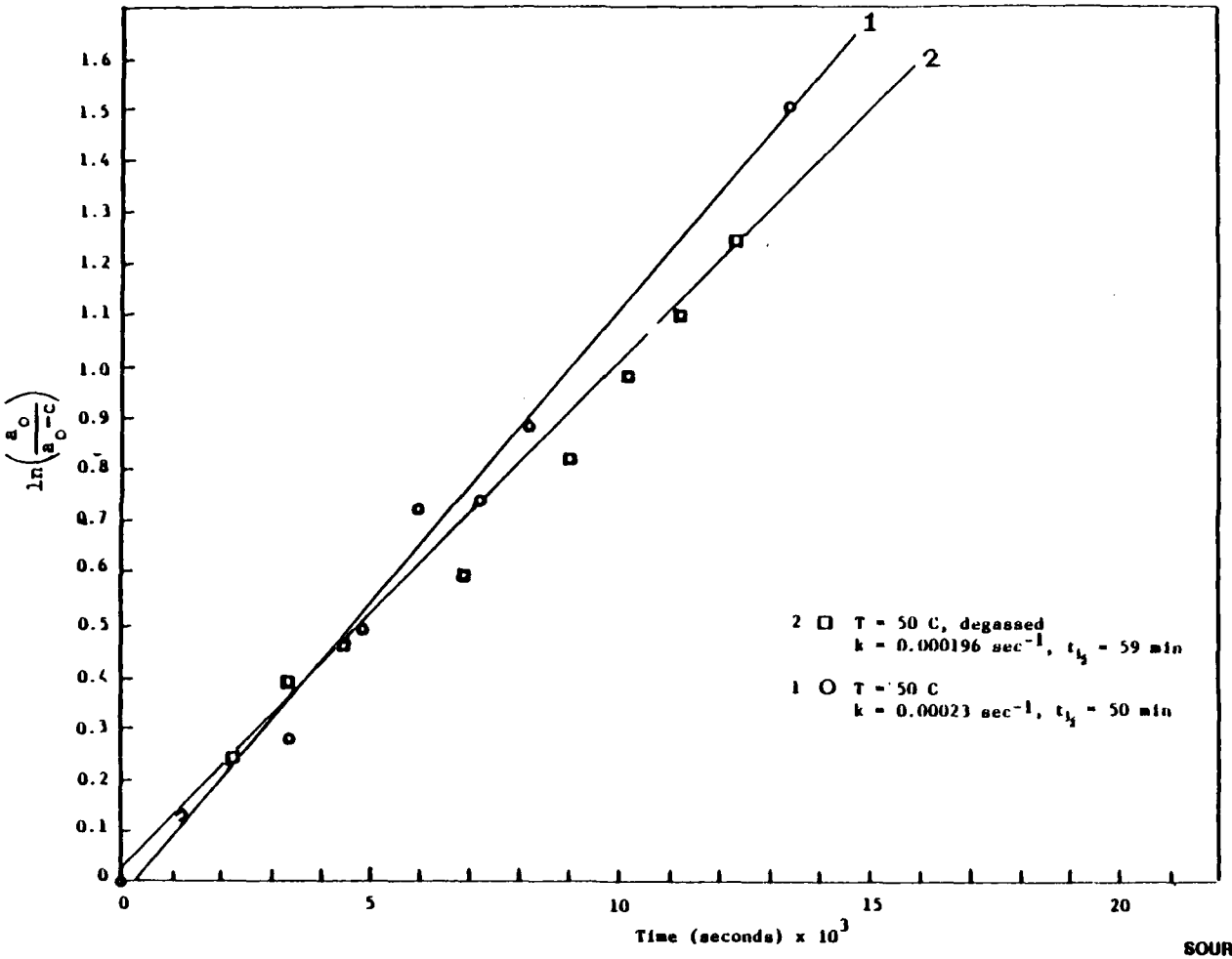

SOURCE: ESE, 1982.

Figure 3-20

RATE OF UDMH DEGRADATION IN DEGASSED AND NONDEGASSED WATER AT 50°C

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Curve 1 is the least-squares regression line resulting from a plot of the 50°C data in nondegassed solution, assuming first-order decay kinetics. Comparison of the rate constant at 50°C with the rate constant at 30°C (Figure 3-19) indicates a 3.25-fold increase in the degradation rate at 50°C. Comparison of the degassed and nondegassed data at 50°C shows a small decrease in the degradation rate in the degassed sample. Statistical evaluation of the data has shown that the slopes of Curves 1 and 2 are statistically different at the 95-percent confidence level. Assuming that the degradation process is due to a combination of hydrolysis and oxidation by reaction with dissolved oxygen, an estimate of the percent of degradation due to hydrolysis can be calculated as follows:

$$K_{ox} + K_{Hyd} = K_{observed\ (Curve\ 1)} = K_1$$

$$K_{Hyd} = K_{observed\ (Curve\ 2)} = K_2$$

$$\%\ (Hydrolysis) = \frac{K_2}{K_1} \times 100\% = 85.2\%$$

$$\%\ (Oxidation) = (100\% - 85.2\%) = 14.8\%$$

3.5.5 Preliminary Experiments and Method Optimization--Soil

The anticipated approach to the analysis of soil for UDMH was to rely heavily on the documented water procedure. Soil samples spiked with or containing UDMH were to be extracted with water and the water analyzed directly for UDMH. Preliminary experiments involving spiking UDMH at approximately 10 ug/g into standard soil followed by extraction with pH-7 buffer water yielded extremely erratic recoveries. Further investigation revealed that soil samples spiked with UDMH in aqueous solution had tc be extracted immediately after spiking if adequate recovery of the spiked UDMH was desired. In addition, significant

loss of UDMH was noted if, during extraction of the soil sample in a 50-ml capped centrifuge tube, the headspace in the tube was not purged with nitrogen to remove the potential for air oxidation. By incorporating rapid sample extraction, rapid purging of the extraction tube with nitrogen, and rapid analysis of the extract, a tentative procedure for UDMH soil analysis was developed. The average recovery of spikes in the 1- to 10-ug/g range was 84 percent if the samples were analyzed immediately after spiking. The following procedure was developed:

1. Accurately weigh 5 grams of the soil sample into a 50-ml centrifuge tube with a screw cap. Record the exact weight.
2. Add 15 ml of phosphate-buffered water (pH = 7), and purge the container headspace with nitrogen for approximately 30 seconds (HPLC-grade water must be used).
3. Seal the centrifuge tube and wrap with aluminum.
4. Shake on a wrist-action shaker for 5 minutes.
5. Centrifuge for 5 minutes at 3,000 rpm.
6. Decant the supernatant liquid into a clean 50-ml volumetric flask.
7. Repeat Steps 2 through 6 with an additional 15 ml of phosphate-buffered water.
8. Adjust the extract volume to 50 ml with phosphate-buffered water (pH = 7).
9. Filter approximately 5 ml of this solution through a 0.45-um filter.
10. Transfer exactly 1.0 ml of this filtered solution to a calibrated centrifuge tube.
11. Add 1.0 ml of filtered acetonitrile and mix thoroughly.
12. Inject 250 ul onto HPLC and analyze according to the UDMH water method chromatographic conditions.

No formal documentation of this method was performed by agreement with USATHAMA because of the unstable nature of UDMH on soil matrices and the special spiking procedures required to get good recovery. Results from one set of spiking experiments yielded a Hubaux and Vos (1970) detection

limit of 1.5 ug/g. If the standard USATHAMA-prescribed spiking procedure was followed and the spiked sample was allowed to stand for 1 hour before extraction to allow the spiking solvent to air dry, severe loss of UDMH resulted.

3.5.6 Soil Stability Studies

To document the stability of UDMH on soil matrices, several experiments were conducted. The results indicated that UDMH rapidly degrades in moist soil and is not expected to persist in the environment. These experiments and results are presented in this subsection.

Air-dried standard soil was used as the spiking medium for these studies. Approximately 5 grams of sieved soil (30-mesh) was spiked with 1 ml of freshly prepared phosphate-buffered water (pH = 7) containing 58.8 ug of UDMH; therefore, the spiked level was 11.8 ug/g. Two types of experiments were conducted in duplicate for 1-hour and 24-hour periods. The results of these experiments are summarized in Table 3-14. The analysis procedure employed is described in Section 3.5.5.

Three experiments were conducted further with a 1-hour delay between the time the soil was spiked and sample analysis. The first of these experiments followed the standard USATHAMA spiking protocol. The 1-ml spiking volume was just sufficient to wet the 5-gram soil sample. This mixture was allowed to stand in an uncapped glass container for 1 hour before analysis. The percent UDMH recovery from these experiments was approximately 10 percent. Because UDMH is fairly volatile, with a boiling point of 64°C, this experiment was duplicated in a sealed container to eliminate vapor [illegible] losses. Recovery from the second set of experiments was less than 30 percent. During studies of the kinetics of decomposition of UDMH in water, it was observed that the rate of decomposition of UDMH in deoxygenated water is lower than in air-saturated water. The third experiment consisted of spiking the UDMH onto the soil, purging the container headspace with nitrogen, and

Table 3-14. UDMH Soil Stability Studies

Condition	Hours Before Analysis	Percentage Recovery
Open Container	1	8.6 9.4
Sealed Container	1	30 25
N_2 Purged and Sealed	1	41 37
Sealed Container	24	<4 <4
N_2 Purged and Sealed	24	<4 <4

Source: ESE, 1982.

analyzing after 1 hour in the sealed container. This experiment yielded recoveries of approximately 40 percent.

The results of these three experiments indicated that the decomposition of UDMH in moist soil is considerably faster than in water. The water kinetics studies indicated that the 30°C half-life of UDMH in pH-7 water was 163 minutes. After 1 hour in water, approximately 75% of the UDMH should remain; however, less than 50% of the UDMH remained after 1 hour of contact with the soil when adjusted for recovery. When UDMH was spiked onto the soil and analyzed immediately, the recoveries for UDMH averaged about 85 percent.

Two other experiments were performed with a 24-hour delay between spiking and analysis. In both experiments, sealed containers were used; one container was purged with nitrogen before sealing, and the other contained normal laboratory air. In both experiments, the UDMH concentration decreased to below detectable limits.

In summary, the results of these five experiments indicated that while UDMH is extractable and detectable at the ug/g level in soil, the analysis must be performed immediately after the contamination has occurred to provide meaningful results because, based on these preliminary results, UDMH is not persistent in soil matrices, especially in the presence of water.

3.6 CYCLOTETRAMETHYLENE TETRANITRAMINE (HMX)

3.6.1 Physical and Chemical Properties

The physical and chemical properties of HMX are presented in Section 4.1 in the discussion of the multiple-analyte procedures for RDX, HMX, and PETN.

3.6.2 Results of Literature Search for Analytical Methods

A summary of the literature search results for analytical methods for HMX is presented in Section 4.1.

3.6.3 Preliminary Experiments and Method Optimization--Water and Soil

The development of a single-analyte analytical procedure for HMX in water and soil was expedited by prior development work on the multiple-analyte procedure for HMX, PETN, and RDX as described in Section 4.1. As the extraction, cleanup, and chromatographic conditions were optimal for quantitative analysis of HMX in water and soil, only one minor modification was made in the multiple-analyte procedure to further optimize it for HMX analysis. The UV detector wavelength was changed to 230 nm from the 215-nm wavelength used in the multiple-analyte method. This wavelength (230 nm) corresponds to the maximum UV absorbance of HMX and is favored because of the increased sensitivity for HMX at this wavelength and the lessened chance of detecting UV absorbing interferents at 230 nm as compared to 210 nm. All other conditions were equivalent to the multiple-analyte procedure.

3.6.4 Method Documentation

Documentation of the developed HMX single-analyte procedure was performed according to the project QC Plan. Standard and natural water was spiked at 0, 0.5 DL, 1 DL, 2 DL, 5 DL, and 10 DL, where DL is 1.27 ug/L. For standard and natural soil, the DL level was 1.6 ug/g. The spiked samples were analyzed according to the fully developed procedure, and the detection limit was calculated from the found-versus-target concentration data using the method of Hubaux and Vos (1970). Detection limits of 29 ug/L and 0.98 ug/L were obtained for the standard and natural water matrices, respectively. The detection limit was 4.8 ug/g for standard soil and 4.7 ug/g for natural soil. The precision and accuracy of the method were determined from the slope and standard error of the estimate ($S_{y.x}$) of the found-versus-target concentration data plot. No particular problems were encountered during documentation. The accuracy of the method in natural water over the spiking range of 0.63 to 12.7 ug/L was 81 percent. The accuracy in natural soil over the spiking range of 0.79 to 15.8 ug/g was 77 percent. The average percent imprecision was 5 percent for natural water and 15 percent for natural soil.

3.7 DIPHENYLAMINE

3.7.1 Physical and Chemical Properties

Some of the physical properties and alternate nomenclature for diphenylamine (DPA) are given in Table 3-15. Its chemical structure is illustrated in Figure 3-11. DPA is a secondary amine which exhibits very weak basic properties and has a pK_b of 6 $x10^{-14}$. In natural aqueous systems within the range of pH 4 to 8, DPA exists in the nonionic molecular form. DPA is very soluble in benzene, ether, and chlorinated organic solvents such as chloroform and methylene chloride. It is also highly soluble in ethanol and isopropyl alcohol. Its solubility in water is very low. DPA is extractable from water using toluene or methylene chloride.

3.7.2 Results of Literature Search for Analytical Methods

An extensive literature search for DPA analytical methods was not conducted. A previously developed qualitative screening procedure for DPA in soil had been developed by ESE under previous contracts for USATHAMA. This method, USATHAMA Method DPA 1S, was used as the basis for further methods development work.

3.7.3 Preliminary Experiments and Method Optimization--Soil

The starting point for methods development efforts was USATHAMA method DPA 1S, which had a DPA detection limit of 30 ug/g in standard soil. Experimental efforts concentrated on the lowering of the detection limit of this method to the 1- to 5-ug/g range in soil. Method DPA 1S, a semiquantitative method for aniline, N,N-dimethylaniline, and diphenylamine in soil, employed an extraction with a combined basic water/methanol/toluene-extracting solvent and analysis of the toluene final extract by GC with a nitrogen-phosphorus-specific detector. A chromatogram of the separation obtained using this method (1S) and a summary of the chromatographic conditions are shown in Figure 3-21 and Table 3-16.

Table 3-15. Alternate Nomenclature and Physical and Chemical Properties of DPA

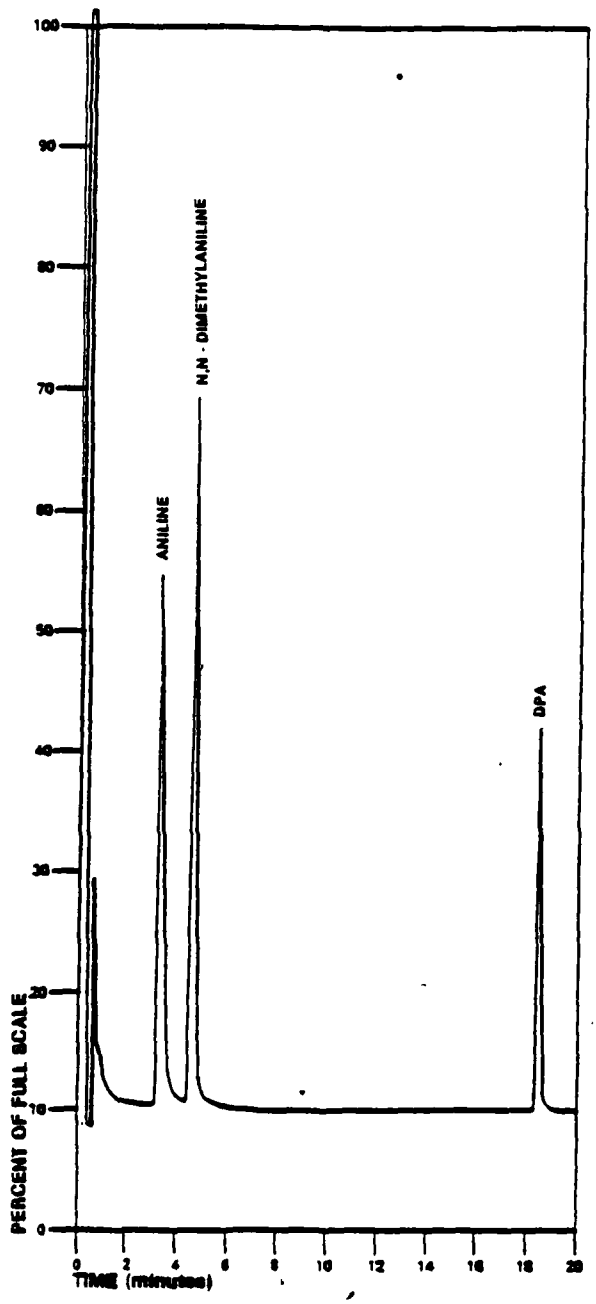
A. ALTERNATE NOMENCLATURE AND CAS REGISTRY NUMBER

Analyte	Alternate Nomenclature	CAS Registry Number
DPA	--	122-39-4

B. PHYSICAL AND CHEMICAL PROPERTIES

Analyte	Formula	Melting Point (°C)	Boiling Point (°C)	Density (g/ml at 20°C)
DPA	$C_{12}H_{11}N$	52 - 54	302	1.16

Source: ESE, 1982.



SOURCE: ESE, 1982.

Figure 3-21

CHROMATOGRAM OF ANILINE, N,N-DIMETHYLANILINE, AND DPA BY USATHAMA METHOD1S

Prepared for:

U.S. Army Toxic and Hazardous Materials Agency

Aberdeen Proving Ground, Maryland

Table 3-16. Chromatographic Conditions for Organic Base Analysis Using a Nitrogen-Phosphorus Detector

Column	Flow Rate	Oven Temperature Program	Injector Temperature	Detector Temperature	Compounds Detected
6-foot x 1/4-inch OD x 2-mm ID glass column 5% SP-2401 DB on 100/120-mesh Supelcoport	Carrier - N_2 - 25 ml/min Detector Air - 100 ml/min Detector H_2 - 5 ml/min	90°C, hold for 8 min; 8°C/min to 170°C; hold for 8 min	250°C	300°C	Aniline N,N-Dimethylaniline DPA

Source: ESE, 1982.

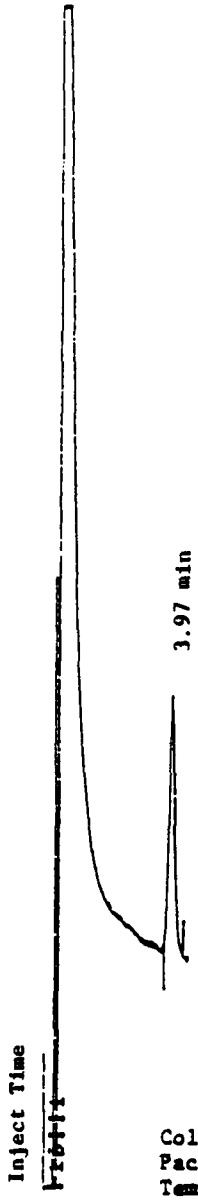
Initial work focused on the optimization of chromatographic conditions for DPA. Because aniline and N,N-dimethylaniline were not to be analyzed by this procedure, the necessity for the long temperature programmed run was eliminated, thereby shortening the analysis time to about 5 minutes. The use of a higher column temperature and a less retentive stationary phase reduced the retention time for DPA and improved the instrumental sensitivity for this compound. The optimum chromatographic conditions consisted of the following:

1. Column: 10 ft by 1/4-inch OD by 2-mm ID, glass, packed with 1.5% OV-17 on 80/100-mesh Supelcoport;
2. Column temperature: 160°C isothermal; and
3. Carrier flow of 50 ml/min N_2.

These conditions resulted in a retention time of 4.0 minutes for DPA. A chromatograph of DPA, spiked into standard soil at 3 ug/g using the optimized DPA chromatographic conditions, is presented in Figure 3-22.

Extraction and concentration of DPA from soil were performed according to the procedure described in USATHAMA Method 1S. The procedure consists of extracting 10 to 15 grams of soil or sediment with a mixed solvent consisting of 20 ml of 0.1 N sodium hydroxide, 10 ml of toluene, and 5 ml of methanol in a capped 50-ml centrifuge tube. The methanol in the solvent is added to improve the mixing of the toluene with wet sediments. The extraction is repeated three times by shaking the centrifuge tube followed by centrifugation to separate the layers. The toluene layers are combined and directly analyzed by GC on an NP detector. Initial experiments showed better than 80-percent recovery of DPA at spiking levels as low as 1.3 ug/g, and, therefore, full documentation was initiated in standard and natural soil.

Method documentation was performed by spiking and analyzing standard and natural soil at 0, 0.5 DL, 1 DL, 2 DL, 5 DL, and 10 DL, where DL is 2.6 ug/g. The detection limit of the method in natural and standard soil was calculated by the method of Hubaux and Vos (1970) from the



Column: 10-ft x ¼-in OD x 2-mm ID glass
Packing: 1.5% OV-17 on 80/100-mesh Supelcoport
Temperature: 160° C
Carrier Flow: 50 ml/min N_2
Detector: Nitrogen-phosphorus, rubidium bead

SOURCE: ESE, 1982.

Figure 3-22

CHROMATOGRAM OF DPA SPIKED AT 3 ug/g INTO STANDARD SOIL

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Aberdeen Proving Ground, Maryland

least-squares regression line of the found-versus-target concentration data. The detection limit in standard soil was 1.5 ug/g and 1.6 ug/g in natural soil. Precision and accuracy data were generated from the slope and standard error of the estimate of the least-squares regression line of the found-versus-target concentration data. No difficulties were encountered during method documentation. The accuracy of the method in natural soil over the spiking range of 1.5 to 29.7 ug/g is 99 percent. The average percent imprecision for natural soil was 4.8 percent.

4.0 MULTIANALYTE METHODS DEVELOPMENT

4.1 RDX, HMX, AND PETN

The following section describes the approach taken for the development of a multiple-analyte analytical procedure for cyclotrimethylene-trinitramine (RDX), cyclotetramethylenetetranitramine (HMX), and pentaerythritol tetranitrate (PETN). A multiple-analyte method for these compounds was appropriate for several reasons, including: (1) the chemical similarity of RDX, HMX, and PETN; (2) the likelihood of finding HMX in RDX-contaminated natural samples; and (3) the similar HPLC conditions including HPLC column, mobile phase, and monitoring UV wavelength required for the analysis of these analytes.

4.1.1 Physical and Chemical Properties

Some of the physical properties and alternate nomenclature for RDX, HMX, and PETN are listed in Table 4-1. The chemical structures of these compounds are presented in Figure 4-1. RDX and HMX are the tri-nitrated and tetra-nitrated derivatives of cyclic tri- and tetra-methylene. HMX is found as an impurity in the manufacturing process for RDX and vice versa. RDX-contaminated environmental samples of water and soil are likely to contain HMX as a co-contaminant.

The UV absorption spectra of HMX and RDX are similar; both possess an absorbance maximum near 230 nm. Their spectra also display a weakly absorbing tail extending into the UV-B spectral region (280 to 320 nm). The absorption spectrum of PETN has very weak oscillator strengths for wavelengths longer than 220 nm and has a fairly strong end absorption beginning at about 220 nm and extending to shorter wavelengths in the vacuum UV.

Hydrolysis of HMX, RDX, and PETN does not occur readily at natural pH conditions. However, RDX and HMX undergo significant hydrolysis under highly alkaline conditions. The hydrolysis of PETN is temperature dependent and is strongly accelerated by both acids and bases.

Table 4-1. Alternate Nomenclature and Physical and Chemical Properties of PETN, HMX, and RDX

A. ALTERNATE NOMENCLATURE AND CAS REGISTRY NUMBER

Analyte	Alternate Nomenclature	CAS Registry Number
PETN	Pentaerythrite tetranitrate Pentaerythritol tetranitrate 2,2-Bis[(nitrooxy)-methyl]-1,3-Propanediol dinitrate (ester) Nitropentaerythritol Pentrit	78-11-5
HMX	Cyclotetramethylenetetranitramine Octahydro-1,3,5,7-tetrazocine 1,3,5,7-Tetranitro-1,3,5,7-tetrazacyclooctane Octogen	2691-41-0
RDX	Cyclotrimethylenetrinitramine Hexogen, T-4, Cyclonite, Hexahydro-1,3,4-trinitro-s-triazine	121-84-4

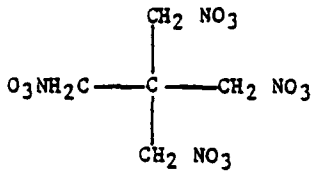
B. PHYSICAL AND CHEMICAL PROPERTIES

Analyte	Formula	Water Solubility (mg/L)	Melting Point (°C)	Boiling Point	Density (g/ml)
PETN	$C_5H_8O_{12}N_4$	43 (25°C)	141	180 at 50 torr	1.77
HMX	$C_4H_8O_8N_8$	140 (83°C)	276	--	1.77 - 1.96*
RDX	$C_3H_6O_6N_6$	76 (25°C)	204.1	--	1.816

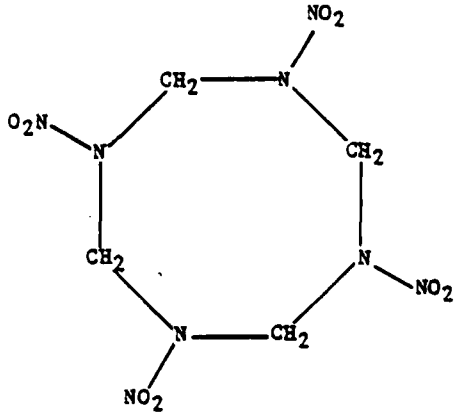
* There are four polymorphic forms of HMX with this range of densities.

Sources: Small and Rosenblatt, 1974.
ESE, 1982.
Merck Index, 9th Edition, 1976.

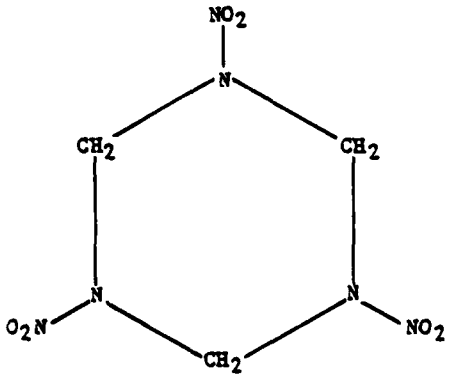
PETN



HMX



RDX



SOURCE: ESE, 1992.

Figure 4-1

CHEMICAL STRUCTURES OF PETN, HMX, AND RDX

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U.S. Army Toxic and Hazardous Materials Agency
Aberdeen Proving Ground, Maryland

4.1.2 Results of Literature Search for Analytical Methods

Prestia (1975) suggested that polarography using a dropping mercury electrode system could be used to quantitate RDX and HMX in environmental samples. Whitnack (1976) developed a technique (single-sweep polarography) which could analyze RDX or HMX at the 0.05-mg/L level in a 2-ml aliquot of effluent.

TLC on silica-gel plates has been used for RDX and HMX analysis in water and sediments. Leach and Hash (1972) reported detection limits for RDX of 25 mg/L and 40 mg/L by direct determination using TLC. Using TLC, a detection limit for HMX of 1 mg/L for sediment or water samples was reported by Sullivan et al. (1977). Jain (1976) quantitated HMX in wastewaters used in adsorption experiments by TLC. Munitions components such as RDX, TNT, HMX, and photolysis products have been separated from extracts for quantitative identifications by TLC (Hale et al., 1978; Stilwell et al., 1977; Sikka, 1978; Jackson et al., 1976).

Gas-liquid chromatography using EC or flame ionization detection has been routinely used to measure RDX in water, sediment, and soil samples contaminated with munitions residues (Hoffsommer et al., 1977; Jain, 1976; Sullivan et al., 1977; Bentley et al., 1978). PETN has been determined by Yinon (1980) by negative ion chemical ionization mass spectrometry, a highly sensitive and specific analytical technique. Davidson et al. (1971) obtained a GC separation and detection of the pentaerythritol nitrates with a sensitivity in the submicrogram range using flame ionization detection, and a sensitivity in the nanogram to subnanogram range with EC detection.

HPLC has often been used for the determination of PETN because of the selectivity of its detectors. Lafleur and Morriseau (1980) used HPLC with a nitrosyl-specific detector for the determination of explosives at trace levels. Krull and Camp (1980) and Fine et al. (1975) mention the use of the thermal energy analyzer (TEA) for trace determination of N-nitroso compounds.

HPLC using an electrochemical detector in the reductive mode using a single-electrode amperometric transducer with glassy-carbon and Au/Hg electrodes has been used to determine reducible explosives in water samples (Bratin and Briner, 1980). Difficulties in this technique include interference from dissolved oxygen and trace metals.

4.1.3 Preliminary Experiments and Method Optimization--Water

The emphasis in this project was placed on the development of a GC or HPLC technique using commonly available detectors. Techniques such as polarography, GC/MS, and HPLC with expensive or less common detectors were not selected for further development.

The major drawback to the use of GC for analysis of RDX, HMX, and PETN is that only RDX and PETN can be analyzed because HMX is not sufficiently volatile and stable to be chromatographed in this manner. HPLC overcomes the problems of thermal stability and low volatility which hinder GC analysis. Analysis time for HPLC is rapid; a sample can be analyzed in 15 minutes (Stilwell et al., 1977). Using careful analytical techniques, HPLC afforded the best analytical system for routine monitoring of trace concentrations of RDX, PETN, and HMX in environmental samples. Further justification for the selection of an HPLC approach to method development is the existence of a previously developed HPLC method for the quantitative analysis of RDX in water (USATHAMA Method 2B). This existing method was readily modified to include the analysis of PETN and HMX. Because RDX could be a potential interference in the analysis of HMX and PETN, initial efforts were directed toward selection of optimum chromatographic conditions appropriate for the separation of PETN, HMX, and RDX. The instrumentation employed was an Altex Model 322 dual-pump liquid chromatograph equipped with a Perkin-Elmer LC-75 variable-wavelength detector.

The existing analytical method for RDX utilized a methylene chloride extraction of RDX from water samples. Considering the similarity in structure between RDX and HMX, methylene chloride was the logical choice

as the extraction solvent for the multiple-analyte methodology. The extraction characteristics of PETN were unknown prior to the initial development experiments; however, methylene chloride liquid/liquid extraction also appeared to be the simplest and quickest means of isolating PETN. Because PETN has significant UV absorption at wavelengths less than 220 nm, the detection wavelength (215 nm) for this method was chosen to provide adequate sensitivity for PETN. Both HMX and RDX absorb strongly at 215 nm.

The retention behavior of the three compounds on two chromatographic stationary phases is presented in Table 4-2. The chromatography columns were an Ultrasphere-ODS (4.6-mm ID by 25 cm), a C-18 bonded packing material with a mean particle size of 5 um, and Zorbax-CN (4.6-mm ID by 25 cm), an alkylcyano bonded packing material with a mean particle size of 7 to 8 um. Both of these columns were operated in the reversed-phase mode. It was observed that the elution order of HMX and RDX could be reversed on the ODS column when the mobile phase was changed from 50% acetonitrile/50% water to 50% methanol/50% water. A similar elution order reversal of HMX and RDX also was observed using 50% methanol/50% water eluent by changing to the Zorbax-CN cyano-phase column. This reversal indicates that the separation mechanisms on the two types of columns are different. The cyano and ODS columns could act as excellent confirmatory columns in the analysis of an unknown sample for RDX and HMX.

The Zorbax-CN column was selected for the analysis because it separated the three compounds within a reasonable elution time without the necessity for gradient elution. The mobile phase used in this case was 65% methanol/35% water. A summary of the column conditions investigated and the observed retention times is given in Table 4-2.

Because methylene chloride was known to efficiently extract RDX from water, a study was made to determine whether methylene chloride (CH_2Cl_2) could also be used to extract HMX and PETN. Five

Table 4-2. Retention Behavior of RDX, HMX, and PETN

Column	Eluent	R_t (min) RDX	HMX	PETN
Ultrasphere-ODS	$CH_3OH:H_2O$ = 70%:30%	Interfered by solvent peak		4.8
	$CH_3CN:H_2O$ = 50%:50%	5.2	5.4	19.4
	$CH_3OH:H_2O$ = 50%:50%	6.1	3.8	26.7
	Gradient*	6.1	3.7	18.1
Zorbax-CN	$CH_3OH:H_2O$ = 70%:30%	6.8	10.1	10.7
	$CH_3OH:H_2O$ = 65%:35%	7.8	11.5	13.5

* 50% methanol:50% water, hold 5 minutes and increase to 85% methanol: 15% water over 20 minutes.

Source: ESE, 1982.

hundred ml of standard water was spiked with 10 ug/L of RDX, 8.5 ug/L of HMX, and 60 ug/L of PETN. Serial extraction with three 100-ml portions of CH_2Cl_2 was conducted in a 1-liter separatory funnel. The extracts were combined and added to a 500-ml K-D apparatus with a 10-ml graduated receiver. The solution volume was reduced by controlled heating over a water bath, and the CH_2Cl_2 was solvent-exchanged to a final volume of 1 ml of methanol. After the addition of 1 ml of H_2O, the extracts were injected onto the HPLC column and analyzed. The technique of "salting out" to increase the extraction efficiency of these highly water soluble compounds was investigated for two of the samples by adding 100 ml of saturated aqueous sodium chloride to the spiked samples before extraction. The results of these experiments, presented in Table 4-3, indicated that "salting out" had a minimal effect on the extraction efficiency of RDX, HMX, and PETN.

No cleanup step was included in the water method for RDX, HMX, and PETN because no interferences were found in standard or natural water documentation. However, subsequent work on the soil/sediment method included a silica-gel cleanup of these compounds prior to HPLC chromatography. Although this step was not incorporated into the documentation of the water method, the addition of the silica-gel cleanup for complex water samples could easily provide a means of eliminating possible interferences.

The instrumental sensitivity for PETN was 7.5 times less than the instrumental sensitivity of HMX and 5 times less than RDX at a 215-nm monitoring wavelength. Using a 250-ul injection volume, an instrumental sensitivity sufficient to yield a detection limit of 1 to 5 ug/L for each component was achieved.

During the course of the multiple-analyte method documentation, the instrumental response of standards was monitored to check the stability of the RDX, HMX, and PETN standard solutions. These standard solutions were diluted from a stock composite standard developed in acetonitrile.

Table 4-3. Percentage Recoveries of RDX, HMX, and PETN With and Without Added Salt

Compound	Percent Recovery (Salt Added)		Percent Recovery (No Salt Added)	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
RDX	101	95	98	100
HMX	64	61	73	75
PETN	100	95	95	91

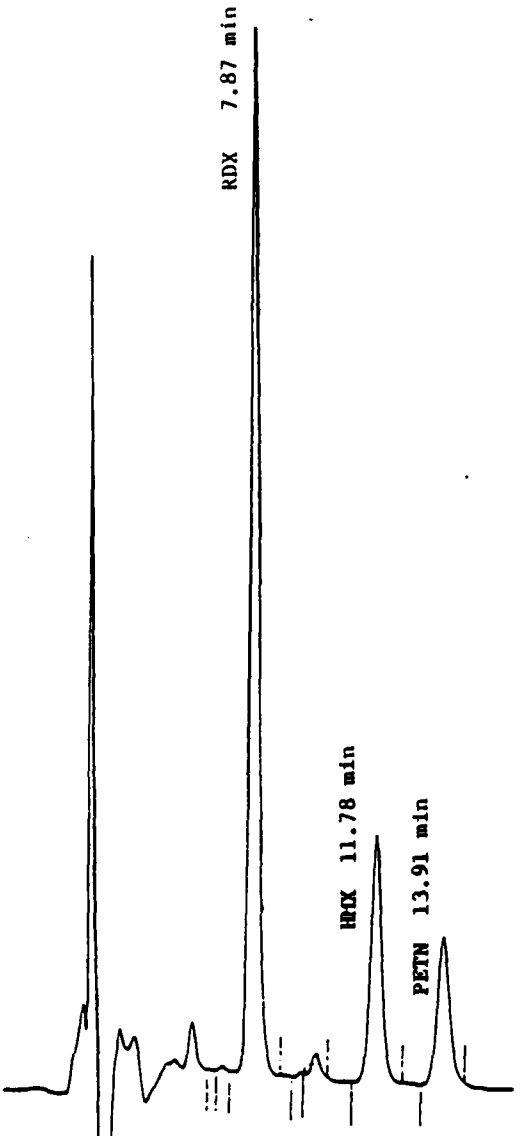
Source: ESE, 1982.

(A small amount of acetone was used to dissolve all three of the analytes before dilution in acetonitrile.) Dilutions for the calibration standards were made in 50% methanol/50% water. These solutions were stored in amber crimp-top vials in a refrigerator each night after use. The standards were chromatographed, and the response in area counts per unit weight injected was calculated on a daily basis. No significant change in instrumental response for the peaks identified as RDX, HMX, and PETN was observed during a 1-month period. A chromatogram of RDX, HMX, and PETN spiked in natural water at 12.6, 4.3, and 15.8 ug/L, respectively, is presented in Figure 4-2.

Method Documentation--Quantitative method documentation was performed in accordance with the project QC Plan. RDX, HMX, and PETN were spiked into standard water and natural water at 0, 0.5 DL, 1 DL, 2 DL, 5 DL, and 10 DL, where DL is 2.52, 0.85, and 3.16 ug/L for RDX, HMX, and PETN, respectively. These spiked samples were analyzed with the entire method. Replicates were analyzed at each spiking level on each of 4 separate days. The detection limit was calculated from the found-versus-target concentration data using the method of Hubaux and Vos (1970). The detection limits for RDX, HMX, and PETN are 1.5, 1.8, and 3.4 ug/L, respectively, in standard water and 4.1, 2.3, and 4.5 ug/L, respectively, in natural water. The precision and accuracy of the method were determined from the standard error of the estimate and slope of the least-squares regression line of the found-versus-target concentration data. The accuracy of the RDX, HMX, and PETN method in natural water was 92% for RDX, 82% for HMX, and 88% for PETN. The average percent imprecision over the spiking range in natural water was 17% for RDX, 19% for HMX, and 26% for PETN.

4.1.4 Preliminary Experiments and Method Optimization--Soil

The approach selected for soil analysis was the application of the previously developed multiple-analyte method for RDX, HMX, and PETN in water to standard and natural soils. Chromatographic conditions had been optimized in the standard and natural water documentation, and



Column: Zorbax-CN (4.6-mm ID x 25 cm, 7 um)
Detector: Perkin-Elmer LC-75 variable-wavelength detector
(λ = 215 nm)
Flow Rate: 1 ml/min
Mobile Phase: 65% methanol/35% water
Injection Volume: 50 ul

SOURCE: ESE, 1982.

Figure 4-2

CHROMATOGRAM OF RDX, HMX, AND PETN IN NATURAL WATER

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Aberdeen Proving Ground, Maryland

there was no attempt to change those conditions for the standard and natural soil documentation. However, efforts had to be made to develop extraction methods for the soil matrix and for the elimination of possible interferences. The existence of a developed RDX-in-soil method (RDX 2C) provided a starting point for the development of an extraction procedure. The soils in this procedure were extracted using water as the extracting solvent. The aqueous extract was relatively free of interferences and was successfully used for RDX analysis without a cleanup step.

An experiment was performed in which HMX and PETN were spiked into standard soil at concentrations ranging from approximately 0.5 to 10 ug/g. These samples were then extracted four times with 35 ml of water exactly as specified in the developed method. The water was then back-extracted three times with 100 ml of methylene chloride, the extract concentrated and solvent-exchanged to methanol. After dilution with water, the sample was analyzed by HPLC. Results of these experiments are given in Table 4-4. These data showed a significant drop in recovery at the 5-DL and 10-DL spiking levels for both PETN and HMX. A possible reason for the loss of these analytes with an increase in concentration could be that HMX and PETN are not sufficiently soluble in the water-extracting solvent at these higher concentrations and, consequently, were not efficiently extracted from the soil.

Based on these results, the use of water as an extraction solvent was not pursued. An alternative plan was developed which involved extracting the soil with methylene chloride containing 20% acetone and cleanup of the extract by silica-gel chromatography. The use of acetone in the extracting solvent was deemed necessary to increase the solubility of the analytes in the extracting solvent and to provide for more effective extraction from wet sediments. Methylene chloride alone would not have the capacity to mix well with a large amount (approximately 10 to 50% W/W) of water as may be found in wet sediments. Because a more nonpolar solvent, methylene chloride, was to be used for

Table 4-4. Recoveries of HMX and PETN from Standard Soil Using Water Extraction Solvent

Level	Amount Spiked (ug/g)	Experiment 1 Amount Recovered (ug/g)	Experiment 1 Percent Recovery	Experiment 2 Amount Recovered (ug/g)	Experiment 2 Percent Recovery
HMX					
Blank	0.0	0.0	0.0	0.0	0.0
0.5 DL	0.532	0.331	62.2	0.440	82.7
1 DL	1.064	0.795	74.7	0.867	81.4
2 DL	2.128	1.57	73.7	1.67	78.4
5 DL	5.32	3.41	64.1	3.65	68.6
10 DL	10.64	2.51	23.5	4.10	38.5
PETN					
Blank	0.0	0.0	0.0	0.0	0.0
0.5 DL	0.395	0.298	75.4	0.357	90.3
1 DL	0.79	0.749	94.8	0.71	89.8
2 DL	1.58	1.26	79.7	1.42	89.8
5 DL	3.95	1.33	33.6	2.92	73.9
10 DL	7.9	0.87	11.0	4.82	61.0

Source: ESE, 1982.

extraction of soils and sediments, the inclusion of a cleanup step in the procedure was necessary to eliminate problems from nonpolar and moderately polar interferent compounds possibly present in the soil and sediment matrices.

An elution scheme for cleanup of the extracts on silica gel was selected from the literature for evaluation. The elution scheme selected was one proposed by EPA for fractionation of a complex extract into fractions of different polarity. The scheme is presented in Table 4-5. The range of eluting solvents corresponded to an increasing level of solvent strength (E°) on silica gel. The elution behavior of RDX, HMX, and PETN under this elution protocol was investigated by performing spiking experiments in standard soil. The soil samples were extracted with 20% acetone/80% methylene chloride, the extracts concentrated by K-D evaporation, solvent exchanged into hexane, and final dilution into 5% methylene chloride/95% hexane. This methylene chloride/hexane fraction was placed at the head of a silica-gel column (10.5 mm by 20 cm) packed with Davidson (600/200 mesh) Grade 950 silica gel and the elution sequence initiated as described in Table 4-5. The silica column was packed in hexane solvent and the column flow rate maintained at 1 ml/min. Fully activated silica gel (activated at 140°C for 2 hours prior to use) was used in this experiment. The results are shown in Table 4-6. No attempt was made to calculate recoveries because only a general elution pattern was desired. It was clear from the elution pattern that PETN, HMX, and RDX, although similar in chemical structure, did not elute in the same column fraction. Considerable overlap of the fractions was also noted.

RDX, HMX, and PETN eluted off the silica gel in solvents greater in polarity than 100% methylene chloride and were completely eluted by 50% methanol/50% methylene chloride. Based on these results, a fractionation scheme consisting of two fractions was selected for the cleanup step. The first column fraction eluted with 20% methylene

Table 4-5. Solvent Elution Sequence for Silica-Gel Cleanup

Fraction	E°*	Solvent Composition	Volume (ml)
1	0.00	Hexane	25
2	0.18	25% methylene chloride in hexane	10
3	0.27	50% methylene chloride in hexane	10
4	0.33	Methylene chloride	10
5	0.53	5% methanol in methylene chloride	10
6	0.63	20% methanol in methylene chloride	10
7	0.68	50% methanol in methylene chloride	10

*E° is the solvent strength on silica gel.

Source: ESE, 1982.

Table 4-6. Elution Behavior of HMX, PETN, and RDX on Fully Activated Silica Gel

Fraction	E°	Solvent Composition	Elution Volume (ml)	Observations		
				RDX	HMX	PETN
1	0.00	100% hexane	25	--*	--*	--*
2	0.18	25% methylene chloride/ 75% hexane	10	--*	--*	--*
3	0.27	50% methylene chloride/ 50% hexane	10	--*	--*	--*
4	0.33	100% methylene chloride	10	--*	--*	(50%)†
5	0.53	5% methanol/ 95% methylene chloride	10	--*	--*	(50%)†
6	0.63	20% methanol/ 80% methylene chloride	10	(80%)†	(20%)†	--*
7	0.68	50% methanol/ 50% methylene chloride	10	(20%)†	(80%)†	--*

* Analyte not observed in this fraction.
† Indicates fractional percentage of total recovered analyte observed in fraction.

Source: ESE, 1982.

chloride/80% hexane containing any possible nonpolar interferences would be discarded, while the second fraction consisting of 50% methanol/ 50% methylene chloride containing all three analytes of interest would be retained. Possible interferences eliminated by this cleanup step include oils, nonpolar and moderately polar aromatics, hydrocarbons, and naturally occurring nonpolar sediment and soil constituents such as humic and fulvic acids.

During later phases of method development, silica-gel Sep-Pak® cartridges were investigated as a possible substitute for the open liquid chromatography silica-gel columns. The two-step fractionation sequence was directly transferable to these cartridges. These cartridges provided the advantages of ease of operation, conservation of materials, and a densely packed, reproducible silica-gel surface. The final method documentation used these Sep-Pak® silica-gel cartridges.

Method Documentation--The fully developed analytical procedure for RDX, HMX, and PETN in soil is presented in Appendix A. Quantitative method documentation was performed by spiking RDX, PETN, and HMX into standard and natural soil at 0, 0.5 DL, 1 DL, 2 DL, 5 DL, and 10 DL, where DL for RDX, HMX, and PETN is 1.9, 1.6, and 1.6 ug/g, respectively. The spiked samples were analyzed with the entire procedure including the silica Sep-Pak® cleanup step. Four sets of spiked samples were analyzed one on each of 4 separate days. The detection limit of the method was calculated from the found-versus-target concentration data using the method of Hubaux and Vos (1970). Precision and accuracy were calculated from the standard error of the estimate and slope, respectively, of the least-squares regression line of the found-versus-target concentration data.

The detection limits of the method were 1.9 and 2.7 ug/g for RDX, 4.6 and 4.1 ug/g for HMX, and 2.4 and 2.3 ug/g for PETN in standard and natural soil, respectively. The accuracy of the method in the natural soil was 98% for RDX, 66% for HMX, and 95% for PETN. The percent

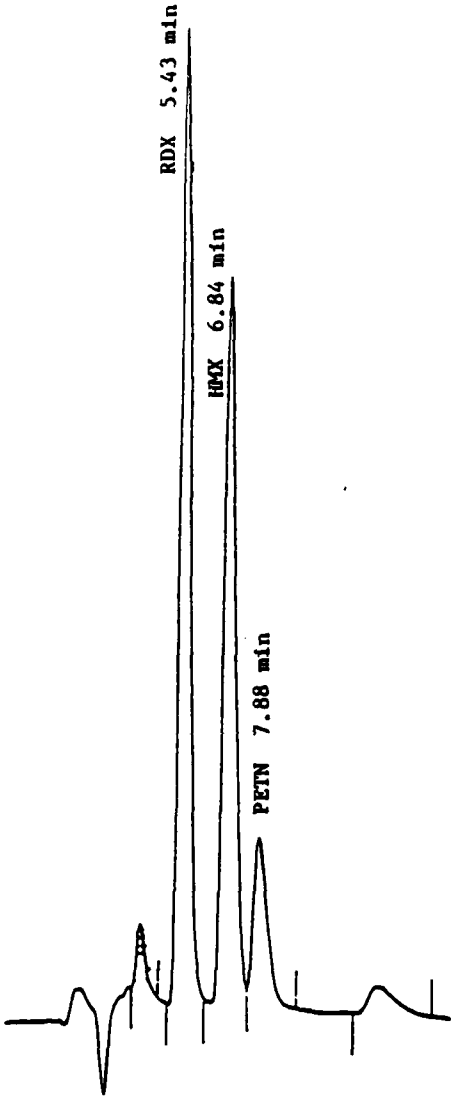
imprecision in natural soil was 13%, 14%, and 18% for RDX, HMX, and PETN, respectively.

No matrix interferences were observed during method documentation. The performance of the HPLC column began to degrade during the documentation analyses. This degradation was evidenced by shortened HPLC retention times for the analytes. Although the column appeared to be less retentive for RDX, HMX, and PETN, adequate separation for these analytes was retained throughout the documentation experiments. A chromatogram of RDX, HMX, and PETN in natural soil obtained under the degraded column conditions is presented in Figure 4-3. Switching columns to a new, Ultrasphere-CN 5-um column restored the original separation. Experiments with this column indicated that this type of column should be stable for at least 3 months with adequate care. No known explanation was available for the column degradation, although the column in question was an old column and had been used frequently previous to this documentation work.

4.2 HPLC SCREEN FOR NITROSUBSTITUTED MUNITION COMPOUNDS AND PAHs

4.2.1 Background and General Summary

The primary objective of this task was the development of an HPLC procedure to simultaneously screen environmental water samples for PAHs and selected munition organic compounds. The analytical literature on HPLC procedures for the quantitation of PAHs is extensive due to the mutagenic, carcinogenic, and teratogenic nature of PAHs. Several HPLC procedures for the analysis of nitrosubstituted munition compounds have also appeared in the recent literature primarily as a result of contract work for the U.S. Army and other DOD agencies; however, no procedures have been developed for the simultaneous screening for both of these classes of compounds in environmental water samples. A further objective of this task was the development of a procedure which provides qualitative information to confirm the identity of unknown analytes, and which can be implemented readily by laboratories equipped with generally available equipment.



Column: Zorbax-CN (4.6-mm ID x 25 cm, 7 um)
Detector: Perkin-Elmer LC-75 variable-wavelength detector
(λ = 215 nm)
Flow Rate: 1 ml/min
Mobile Phase: 65% methanol/35% water
Injection Volume: '50 ul

SOURCE: ESE, 1982.

Figure 4-3

CHROMATOGRAM OF RDX, HMX, AND PETN IN NATURAL SOIL

Prepared for:
U.S. Army Toxic and Hazardous Materials Agency
Aberdeen Proving Ground, Maryland

In many qualitative analytical procedures, the qualitative information is usually generated by one of the spectroscopic methods which include infrared (IR), UV, and MS. The HPLC/IR technique is currently being developed using the Fourier transform (FTIR) technique and shows potential. This method employs normal-phase HPLC for component separation as opposed to the more popular and convenient reverse-phase HPLC due to the broad-band absorbance of water in the IR region. A further complication in this method arises from the IR absorbance of the solvents used in normal-phase HPLC. For these reasons and the expense of the FTIR instrumentation, HPLC/IR was not selected as the separation detection technique for this study.

HPLC/MS was eliminated as a viable alternative because this technique is still being developed and has not yet achieved the sensitivity of other commonly available detectors for HPLC, such as UV and fluorescence detectors. Giles et al. (1979) were able to detect 80 ng of phenanthrene with total ion monitoring and 5 to 10 ng using selective ion monitoring with HPLC/MS, but 0.21 ng using UV detection. These investigators concluded that UV detection "can be used as a relatively inexpensive substitute for the MS in qualitative work." The mass spectra of isomeric PAHs are also very similar [e.g., the mass spectra of perylene, benzo(j)fluoranthene, and benzo(a)pyrene are practically indistinguishable], and additional qualitative information is necessary for identification. Christiansen and May (1978) reported that 1,2-benzanthracene, chrysene, and naphthacene give essentially the same mass spectra and are also difficult to separate.

UV absorption spectroscopy was selected as the method of choice for the qualitative analysis in this study because it is commonly available in most laboratories, it is relatively inexpensive, and it can provide fairly selective information on particular analytes. UV absorption spectra of room-temperature solutions are not usually used for qualitative identification because of the general lack of sharp spectral

bands; however, absorbance ratios at different wavelengths and fluorescence-to-absorbance ratios are fairly selective and have been used in various screening procedures described in the literature.

Of the available HPLC methods for PAHs, Federal Register Method 610 developed by EPA is used widely; however, the only qualitative information generated by this procedure is the chromatographic retention time. Baker et al. (1978) showed that by using relative retention times only 9% of 101 drugs of forensic interest could be distinguished; however, use of a combination of retention times and a single absorbance ratio allowed identification of 95% of the drugs. Giles et al. (1979) used two fixed-wavelength absorbance detectors at 254 and 280 nm for detection of PAHs.

Sorrell and Reding (1979) developed a qualitative screen for 15 PAH compounds using three fixed-wavelength detectors (254, 280, and 340 nm). The absorbance ratios (280/254 nm) and fluorescence-to-absorbance ratios observed by Sorrell and Reding are presented in Table 4-7. For the fluorescence-to-UV ratios, Sorrell used an excitation wavelength of 250 nm, observed emission at wavelengths longer than 340 nm, and used the UV absorbance at 254 nm. The ratio of UV absorbance at two different wavelengths can be combined with retention time data to indicate the presence of a specific compound.

Because many of the PAHs are fluorescent, this means of detection has also been used for enhanced sensitivity and selectivity over absorbance detection. Smillie et al. (1978) used fluorescence-to-absorbance ratios for determination of 10 PAHs in air. The use of fluorescence-to-absorbance ratios in combination with absorbance ratios and retention times enhances the selectivity of the method. Compounds which interfere at one wavelength might not absorb at the second wavelength and a ratio match can then be achieved. Another advantage to the use of fluorescence detection stems from the fact that the majority of nitrosubstituted munition compounds do not fluoresce. An interferent which

Table 4-7. Absorbance and Fluorescence-to-Absorbance Ratios for Selected PAHs

PAH	Absorbance Ratio (280/254 nm)	Fluorescence (>340 nm) / UV Absorbance (254 nm)
Phenanthrene	0.17	0.20
Fluoranthene	1.56	0.61
Pyrene	0.44	NA
Chrysene	0.25	0.41
Benzo(a)pyrene	0.84	1.08
Perylene	0.03	2.38
Dibenzo(a,h)anthracene	6.55	0.38
Indeno(1,2,3-cd)pyrene	0.47	0.35

NA = Not analyzed.

Source: Sorrell and Reding, 1979.

co-elutes with a nitrocompound can be distinguished from the nitrocompound if the interferent also fluoresces.

Several methods have been published for the analysis of nitrosubstituted munition compounds using HPLC. Meier et al. (1978) analyzed for TNT, RDX, and tetryl at the 50-ug/L level by direct injection of as much as 1 ml of water. The detection wavelength in this study was 230 nm. The Midwest Research Institute (1981) developed a procedure for munition organic compounds using a methylene chloride extraction and a detection wavelength of 254 nm. Both of these methods relied solely upon the retention times for qualitative identification of the nitrocompounds. Bratin and Briner (1980) developed an HPLC procedure for RDX, TNT, and nitroglycerin using reductive EC. This procedure was not tested in environmental samples; however, good selectivity and sensitivity for the munition compounds was observed. However, this technique did suffer from the experimental complications inherent in reductive-mode HPLC/EC (e.g., the requirements for thoroughly deoxygenated samples and mobile phase are required).

4.2.2 Selection and Justification of Proposed Approach

The original development approach taken in this task involved the separation of the target compounds into various classes by a combination of solvent extraction, column chromatography, and HPLC using multiple-wavelength absorbance and fluorescence detection. As originally conceived, the method was to involve a base/neutral extraction similar to EPA Method 625 for GC/MS screening of priority pollutants. A base/neutral extraction separates PAHs, munition compounds, and other neutral or basic compounds from acidic species such as 35DNP, other phenolics, and IMPA. This extraction was to be followed by fractionation of the base neutral extract into several portions of varying polarity by column chromatography on silica gel. However, because of the complexity of obtaining a consistent, reliable silica-gel fractionation scheme, in further discussions with USATHAMA, it was decided that the screening procedure would be directed instead toward a more general

cleanup approach that would not involve multiple HPLC analyses of several fractions. The silica-gel cleanup procedure would be employed only for those samples requiring cleanup to eliminate and reduce background interferents. In addition, the silica-gel cleanup would be developed specifically for the compounds of interest and have the minimum number of fractionation steps.

During the initial phase of the task, IMPA was deleted from the target analyte list for two reasons: (1) an analytical method for this compound had already been developed by USATHAMA, and (2) its chemical and physical properties prevented analysis by HPLC/UV. IMPA is a highly water-soluble, ionic species which requires ion chromatography and conductivity detectors for its analysis. IMPA would therefore have to be screened by a different method than HPLC/UV, which was used for the other screen compounds. To extract IMPA from aqueous solutions, a concentration step would be required, possibly using anion exchange resins which would not be applicable to the other compounds on the list.

The deletion of IMPA allowed a simplification of the extraction scheme such that all of the target compounds could be extracted together in one acid/neutral extraction at a pH of 3. To make the screening procedure more pertinent to the type of compounds that might be expected in samples related to military activities, a number of other munition organic compounds were added to the list of analytes including 246TNT, HMX, 135TNB, 13DNB, 26DNT, and 24DNT. The 16 priority pollutant PAHs as defined by EPA were also targeted for study in development of the screening procedure. The additional munition compounds were selected based on the likelihood of finding these analytes in contaminated environmental samples.

The basic scheme developed for the qualitative identification of the targeted compounds is outlined in Figure 4-4. In general, the sample is extracted, concentrated, and divided into two fractions. One fraction is analyzed by HPLC; the other is saved for possible cleanup. If the

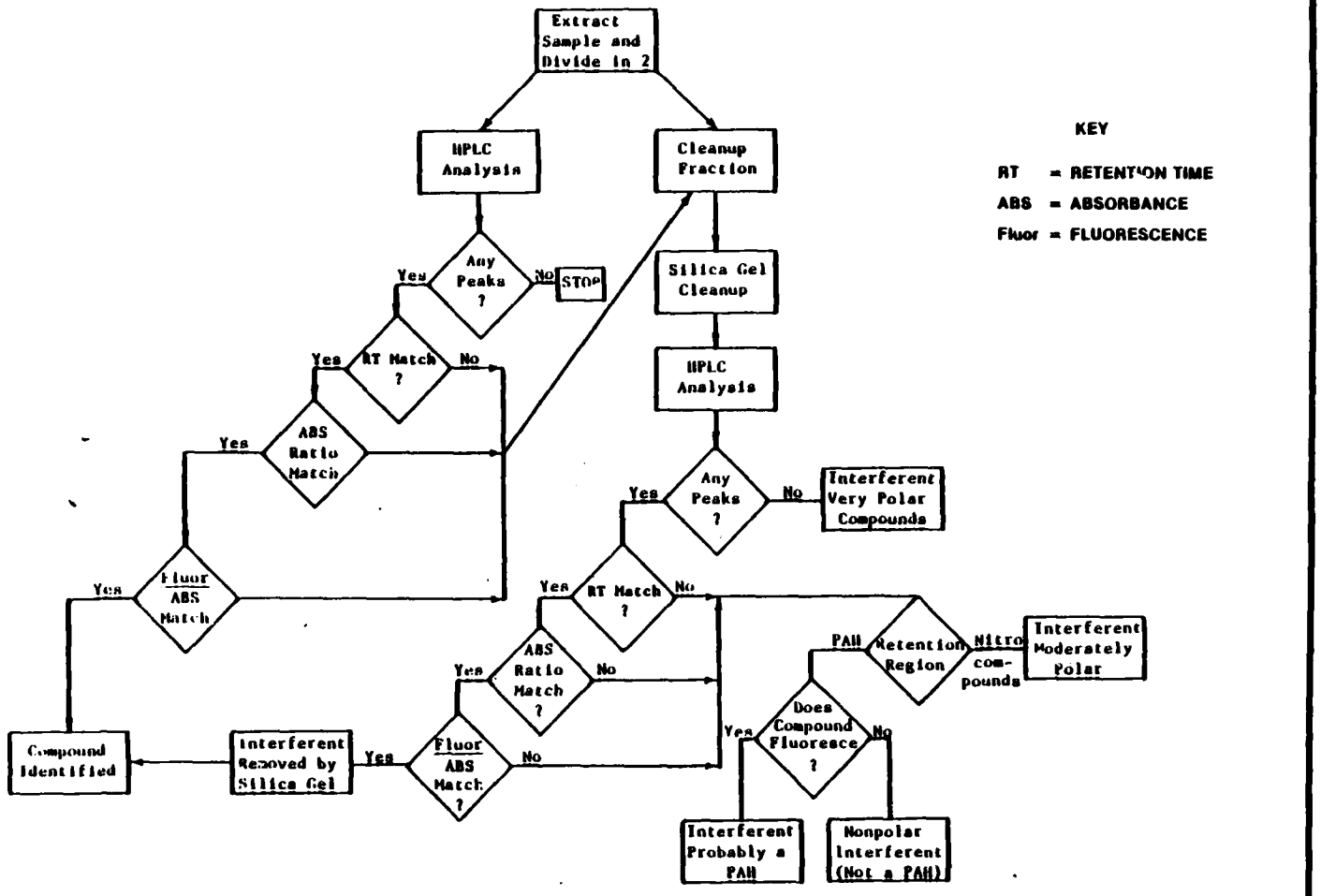


Figure 4-4

LOGIC FLOWCHART FOR QUALITATIVE IDENTIFICATION BY HPLC SCREENING PROCEDURE

Prepared for:

U.S. Army Toxic and Hazardous Materials Agency

Aberdeen Proving Ground, Maryland

retention time and the absorbance or fluorescence ratios for an unknown compound match those for one of the standards, the presence of that particular analyte in the sample is confirmed. If peaks are found for which the retention times match, but for which the ratios cannot be determined because of large amounts of background interferences or do not match any of the standards, silica-gel cleanup is performed on the second sample fraction. The cleaned fraction is then analyzed by HPLC and the same criteria are applied for compound verification.

Extraction of the aqueous sample is performed at a pH of 3 to facilitate the extraction of 35DNP (pK_a = 6.7) (Rochester and Wilson, 1976). Approximately 100 grams of sodium chloride is added to the aqueous sample to aid extraction of the PAHs and munition compounds by the "salting out" effect. The sample is not filtered before extraction because Searl et al. (1979) showed that 90% of the PAHs in natural water samples are associated with the suspended particulates. The sample is then sequentially extracted with three 100-ml portions of methylene chloride. Methylene chloride was selected as the extraction solvent because it has been shown to produce excellent results for PAHs and munition organic compounds and is readily concentrated using standard K-D evaporative/concentrative techniques.

The 300-ml methylene chloride extract is reduced in volume to 20 ml using controlled evaporation in a K-D apparatus and divided into two equal portions. The solvent in one portion is exchanged by multiple evaporations to acetonitrile for subsequent HPLC analysis. The other portion is stored and sealed at 4°C for possible silica-gel cleanup. In this manner, a simple, direct sample preparation is achieved which allows for efficient screening of a number of samples. If during the HPLC analysis of the first portion of the extract no HPLC peaks are detected, the sample can be assumed not to contain any of the analytes of interest, and further analysis is suspended on the sample. If any peaks are detected during this initial screening phase, the retention times and detector ratios for the detected peaks are compared to the

retention times and ratios for the standards. Identification of an unknown compound is verified if the ratios and retention time match those for one of the standard compounds. However, if an unknown peak fails to meet these criteria, cleanup and/or fractionation of the extract followed by HPLC analysis of the second extract portion is conducted. Silica gel is used as the sample cleanup material. Silica will remove polar interferents such as humic and fulvic acids, sulfonic acids, and strong carboxylic acids. Elution of the silica with different polarity eluents can also provide sample fractionation. The compound verification criteria used for the uncleaned sample are applied to HPLC analysis of this cleaned extract. If an unknown compound fails to be verified after sample cleanup and fractionation, the HPLC retention behavior and fluorescence characteristics of the compound can be used to indicate the compound's general classification (see Figure 4-4). The elution time on the HPLC column indicates the relative polarity of the compound. The compound's fluorescent behavior can be used to determine if it could possibly be a PAH compound, because many of the larger PAHs are fluorescent.

Absorbance ratios cannot be employed to unambiguously confirm the absence of a particular compound in a sample extract. If no peak appears in the HPLC chromatogram that matches the retention time of the compound of interest, the compound is definitely absent at levels above the detection limit. However, if a peak does appear in the chromatogram at the correct retention time, there is a definite possibility that the compound of interest is present in the sample, even though the absorbance ratios do not match those for the corresponding standard. This arises from the fact that an interferent compound with different UV absorbance ratios may co-elute at the same retention time as the compound of interest and could modify the measured ratio. On the other hand, a match between ratios and retention times is strong evidence for the presence of the compound.

The use of a silica-gel cleanup step enhances the selectivity of the method by adding another chromatographic separation of the components. Silica gel can be used to fractionate sample components into various groups according to polarities. In this screen, the extracts are placed on a silica-gel Sep-Pak® in methylene chloride. The PAHs and several of the nitroaromatic compounds are not retained on silica gel but are eluted by the methylene chloride; other more polar nitrocompounds such as HMX are retained. The retained nitrocompounds are later eluted with a more polar solvent, 50% methylene chloride in methanol. This fractionation scheme was not optimized to provide separate silica-gel fractions for nitroaromatics and PAHs. There is some overlap of the two types of compounds in each fraction. Emphasis was placed on establishing the recovery, precision, and accuracy of the screening method employing a generalized cleanup procedure rather than developing an exact fractionation scheme. Because no interferents were encountered in the standard and natural waters used for documentation, these fractions were combined during method documentation to expedite chromatography. However, when analyzing real world unknown samples it may be advantageous to analyze the silica-gel fractions separately, which will result in enhanced selectivity due to differences in component polarities. For example, if an interferent occurs in the PAH region of the chromatogram, it is necessary to elute and analyze only the PAHs with methylene chloride. In this manner, many polar compounds can be eliminated from the PAH region of the chromatogram. An important limitation regarding a general sample cleanup, such as that presented in this method, is that it does not necessarily apply to all samples because a priori the type of interferents in any particular sample is not known; therefore, cleanup must be tailored for each sample.

The amount of qualitative information about the sample can be increased through the use of scanning absorbance detectors. The use of stopped-flow scanning of a sample peak can be used to help confirm the presence of a target compound. Recently, detectors have become commercially available which can scan the UV-visible absorbance spectrum

of an eluting HPLC peak without stopping the flow. A complete spectrum can be obtained in less than a second. Because these detectors are not readily available in many laboratories and are rather expensive (>$30,000), the use of absorbance and fluorescence-to-absorbance ratios was selected as the method of choice for the qualitative screening primarily based on the utility with which it can be implemented while recognizing its inherent limitations. The use of comparatively inexpensive detectors also leads to a more cost-effective analysis scheme.

The use of a multidetector approach, which consists of absorbance and fluorescence detectors in series as in this study provides advantages stemming from the fact that often one detector will be much more sensitive to a particular compound than other detectors. This effect can be illustrated for several of the PAHs. Fluorescence is much more sensitive for many of the heavier PAHs than is absorbance detection, and the signal-to-noise ratio for fluorescence is much greater. The desired detection limit for each compound is in the range of 1 to 5 ug/L, and most of the PAHs are detectable at these levels with the absorbance wavelengths used. Therefore, qualitative information can be generated at these levels; however, the PAHs will actually be detectable at much lower levels by fluorescence. This situation is reversed with the munition compounds because generally they do not fluoresce. The fluorescence-to-absorbance ratios of these nitrosubstituted compounds are close to zero. By taking advantage of the varying detector sensitivities of each compound, the most sensitive detector for each compound can be selected to yield the best signal-to-noise ratios for the quantitation. Also, if a compound is detectable by more than one detector, the compound can be quantified by each of the various detectors as a check on instrumental stability and to increase precision.

One of the advantages of using three detectors to generate more than one ratio is illustrated in the following example. If a peak appears in the

chromatogram of a sample at the retention time of one of the target compounds and only one of the ratios matches the target compound, the presence of an interferent is indicated. If the interferent exhibits absorbance at the wavelength that gives the nonmatching ratio and does not absorb at the wavelength that gives the proper ratio, the wavelength for quantitation of the compound can be selected to be the one which has the minimum interference and therefore more accurate value for the analyte.

4.2.3 Preliminary Experiments and Method Optimization

The developmental work was directed toward the following tasks:

1. Assembly and testing of the screening instrumentation,
2. Determination of the optimum HPLC parameters, and
3. Development of a suitable extraction and cleanup procedure.

These tasks are addressed in the following paragraphs, with emphasis on pertinent experimental observations and justifications for the particular strategies and directions taken in the development of this screening procedure.

Task 1--Assembly and Testing of the Screening Instrumentation

Because a comparatively large number of analytes were to be analyzed in a single HPLC run, the resolution of the HPLC column system had to be sufficient to resolve closely eluting compounds. To obtain valid detector ratios, closely eluting bands must have a resolution, R_s, of approximately 1.0 or greater as defined by the fundamental LC relationship (Snyder and Kirkland, 1979).

$$R_s = 0.25.(\alpha - 1).\sqrt{N} \cdot \left[\frac{k'}{1+k'}\right] \qquad \text{Equation 1}$$

Resolution can be maximized by increasing the column selectivity, α, the total number of theoretical plates, N, or the capacity factor, k'.

One of the easiest ways to increase resolution is to increase N by increasing the length of the HPLC column; therefore, two standard HPLC columns (4.6-mm ID by 25 cm) in series were employed. Dual HPLC columns should be approximately additive in the number of theoretical plates when allowance is made for losses in resolution due to band broadening which will occur in the dead volume of the column union. A doubling of the number of theoretical plates results in an increase in the resolution by a factor of 1.4.

The first column configuration tried consisted of two Ultrasphere-ODS, 5-um columns connected in series by a 1-inch section of 0.010-inch, stainless-steel tubing using low-dead-volume fittings. Chromatograms were obtained on a mixture of acetophenone, nitrobenzene, benzene, and toluene in methanol using a 254-nm, fixed-wavelength detector. The toluene peak was used for determining the efficiency of the column system by calculating the number of theoretical plates, N, using the formula:

$$N = 5.54 \left(\frac{R_t}{FWHM} \right)^2$$

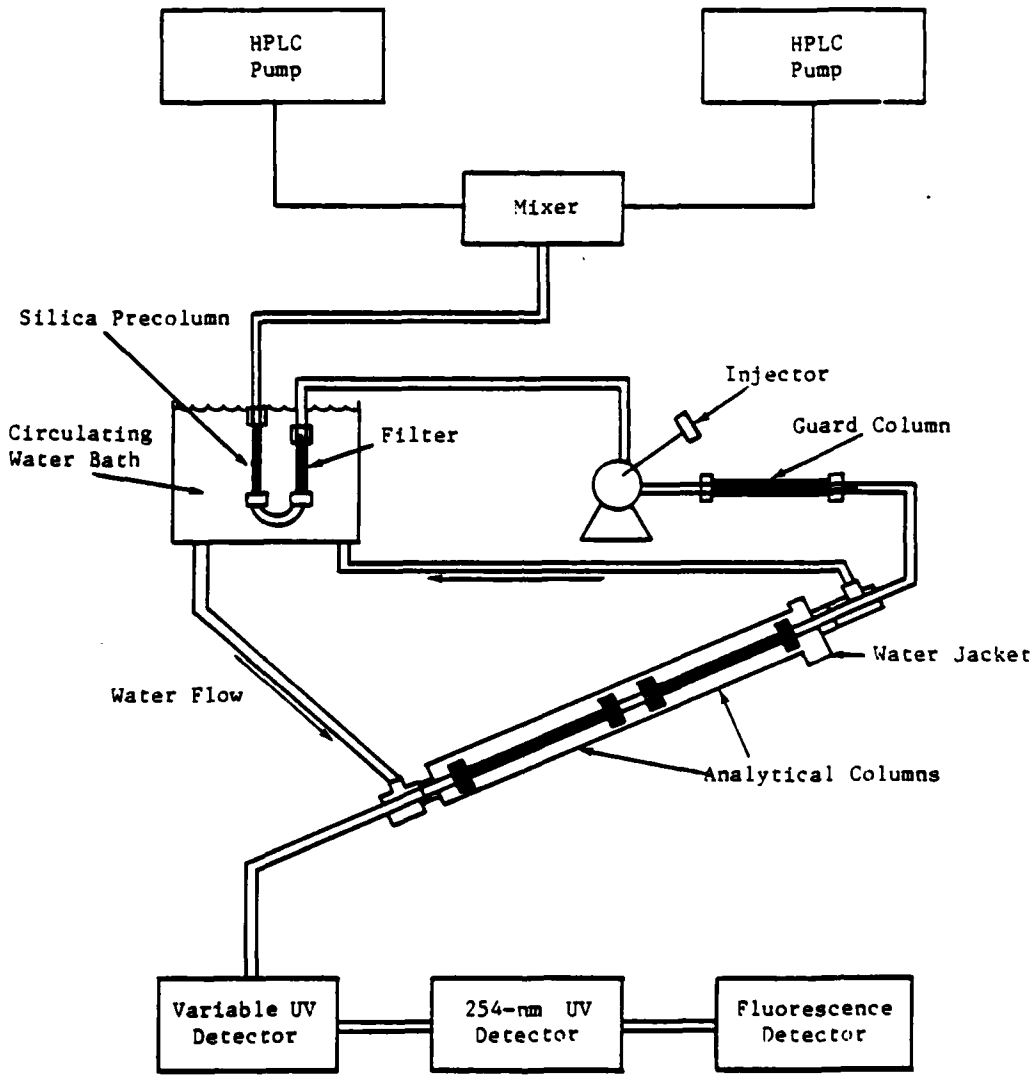
where: R_t = the retention time, and
FWHM = the full width at half-maximum for the peak in the same units as R_t.

For the separate columns, N was determined to be 13,000 and 9,000 plates, respectively, at 22°C using isocratic elution with a flow of 1 ml/min of 50% acetonitrile/50% water. The two columns connected in series gave a plate count of 22,300 plates. Insertion of a guard column (2.1-mm ID by 7 cm) packed with Co:Pell-ODS, 40-um spherical packing yielded a plate count of 21,100 plates, which indicated the addition of a minimum amount of dead volume in the connections and the guard column.

Use of two 25-cm, 5-um particle size columns in series substantially increased the back pressure on the pumping system. At ambient temperatures, the pressure generated from the dual-column system would exceed 7,000 pounds per square inch (psi) using a methanol/water mobile phase and 5,000 psi using an acetonitrile/water mobile phase. This back pressure was significantly reduced by increasing the temperature of the column which decreases the viscosity of the mobile phase. The use of temperature-controlled columns also decreased the variability of analyte retention times due to fluctuations in the ambient temperatures. An advantage of elevated temperature is the decreased retention time for most analytes which leads to shortened analysis times.

A schematic diagram of the HPLC apparatus is presented in Figure 4-5. The two HPLC columns were enclosed in a water jacket (Altech Associates) and a circulating, temperature-controlled water bath (Fisher Scientific Model 80) was used to maintain the jacket temperature. A silica-gel precolumn and 0.25-um filter were inserted between the HPLC pumps and the injector for saturating the mobile phase with silica to extend column life and to filter out any particulates present in the mobile phase. The precolumn and filter were immersed in the water bath reservoir so that silica saturation of the mobile phase would occur at the same temperature as the analytical columns. The guard column between the injector and the analytical column was not heated.

In the preliminary testing of the apparatus, the mobile phase reservoirs were heated to approximately 70°C to avoid any bubble formation in the analytical columns on heating of the mobile phase to the initial column operating temperature of 65°C. Degassing was anticipated due to the decrease in solubility of gases in liquids with increasing temperature. However, in mobile phase reservoirs maintained at ambient temperature, no bubble formation was noted if the mobile phase was thoroughly vacuum-degassed before use. This degassing procedure was followed for subsequent work.



SOURCE: ESE, 1982.

Figure 4-5
HPLC SCREENING APPARATUS

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U.S. Army Toxic and Hazardous Materials Agency
Aberdeen Proving Ground, Maryland

The three detectors were interconnected using 1/16-inch OD by 0.010-inch ID, stainless-steel tubing and low-dead-volume fittings. A Perkin-Elmer LC-75 variable-wavelength spectrometer with autocontrol and scanning capability was the first detector after the column. The 2 feet of post-detector tubing (0.025-inch ID) provided with this detector was changed to 0.010-inch ID tubing to minimize band spreading during transfer to the Altex Model 153 fixed-wavelength, 254-nm detector cell. The effluent from this 254-nm detector was then connected to the inlet fitting of a Perkin-Elmer Fluorescence Spectrometer Model 650-S using less than 3 inches of 0.010-inch ID stainless-steel capillary tubing. Approximately 12 inches of 0.010-inch ID tubing was connected between the inlet fitting and the fluorescence cell. It was very important to make all connections with bored-through unions and/or low-dead-volume fittings.

The Model 650-S fluorescence detector was very useful for further confirmation of compound identity because it was possible to scan excitation, emission, or synchronous spectra; however, less-expensive, filter-type fluorescence detectors may be adequately substituted. Furthermore, a scanning absorbance spectrometer, such as the LC-75, possesses a distinct advantage in compound confirmation because an entire spectrum of an unknown sample component can be obtained; however, any detector capable of automatically or manually switching between two preset wavelengths may be substituted. The recently available dual-wavelength detectors, which simultaneously monitor at two wavelengths, can be good substitutes.

The entire analytical system was automated as completely as possible to expedite the method documentation process. An Altex Model 500 autosampler was used for sample injection and each detector was connected to a Spectra Physics Model 4100 integrator. An Altex Model 420 microprocessor was used to control the pumps and signal the variable-wavelength detector. The microprocessor initially signals the autosampler to load the sample loop. The autosampler then flushes the

sample loop for 60 seconds, injects the sample onto the analytical column, and signals the three integrators to start. After 10 minutes, the microprocessor sends a second flag to the autosampler to reset it prior to the next injection. After 60 minutes, the microprocessor signals the Perkin-Elmer LC-75 with autocontrol to switch detection wavelength from 230 to 280 nm. This wavelength is reset to 230 nm by another signal from the microprocessor, and a second signal to the LC-75 resets the detector for the next injection at the end of each run.

Task 2--Determination of the Optimum HPLC Parameters

This task was directed toward selecting the chromatographic conditions for optimum resolution of the target compounds and confirmation of the component elution order.

Once a dual-column approach had been selected to maximize efficiency, it was necessary to select the type or combination of column packing material which gives the best resolution. Referring to Equation 1, the value of N was optimized by using two columns. The other factors which can influence resolution are the capacity factor, k', and the selectivity factor, α. The optimum range for k' is approximately 1 to 10. Values greater than 10 are unavoidable if many compounds are to be resolved. In the final method, the k' values for the munition compounds were maintained at less than 10 and the PAHs at less than 20. The selectivity parameter is a function of the mobile and stationary phase compositions. This parameter was optimized by trying various combinations of solvents and elution programs and different column packings. Methanol/water and acetonitrile/water mobile phases in different combinations were tried under various elution conditions with two Ultrasphere-ODS, 4.6-mm by 250-mm columns and simultaneously, on separate instrumentation, experiments were conducted on a single Ultrasphere-CN column.

To examine the behavior of the PAHs on these columns, a working standard was prepared from a methanol/methylene chloride solution containing the

16 priority pollutant PAHs. The solvent used was 50% acetonitrile/50% water. The analyte concentrations in this solution are listed as follows:

Priority Pollutant PNA	Concentration (mg/L)
Acenaphthene	10
Fluoranthene	2
Naphthalene	10
Benzo(a)anthracene	1
Benzo(a)pyrene	1
Benzo(b)fluoranthene	2
Benzo(k)fluoranthene	1
Chrysene	1
Acenaphthylene	20
Anthracene	1
Benzo(ghi)perylene	2
Fluorene	2
Phenanthrene	1
Dibenzo(a,h)anthracene	2
Indeno(1,2,3-cd)pyrene	1
Pyrene	1

The best separation for the PAHs was achieved on the dual Ultrasphere-ODS columns at a flow rate of 2 ml/min using a mobile phase of 50% acetonitrile/50% water for 10 minutes, followed by an increase in the acetonitrile percentage to 100% over 30 minutes. The column temperature was 65°C. Under these chromatographic conditions, 15 of the 16 PAHs present in the mixture were resolved.

To examine the elution behavior of the munition compounds, a composite standard of 10 compounds was prepared in 50% acetonitrile/50% phosphate-buffered water (pH = 3) at the following levels:

Compound	Concentration (mg/L)
RDX	10
HMX	10

Compound	Concentration (ug/L)
Tetryl	10
24DNT	10
26DNT	11.4
35DNP	12.1
246TNT	10
13DNB	11
NB	8
135TNB	9.5

A large number of experiments were run on these composite standards using the dual Ultrasphere-ODS column and various experimental conditions:

1. The flow rates were varied from 1.0 to 1.5 ml/minute;
2. Chromatograms were obtained at 40°, 50°, and 65°C; and
3. Methanol, acetonitrile, and combinations thereof were used as the strong elution solvent under various gradient conditions.

The best resolution of the nitrocompounds using the dual Ultrasphere-ODS columns was obtained at 50°C with a flow rate of 1.4 ml/minute. The strong solvent was 12.5% acetonitrile in methanol, and the weak solvent was phosphate-buffered water at a pH of 3. The elution program was held at 30% strong solvent for 10 minutes then increased to 45% strong solvent at 1%/minute. The detection wavelength was 254 nm. This elution program did not provide satisfactory resolution of the 10 target munition compounds.

To summarize the work on the Ultrasphere-ODS columns, adequate resolution was obtained for the PAHs; however, the munition nitrocompounds were not adequately separated. This result is consistent with the expected behavior of ODS-type packing, which is primarily designed for use with nonpolar compounds like PAHs.

Many of the munition nitrocompounds are polar and more water-soluble than PAHs; therefore, the munition nitrocompounds should be better

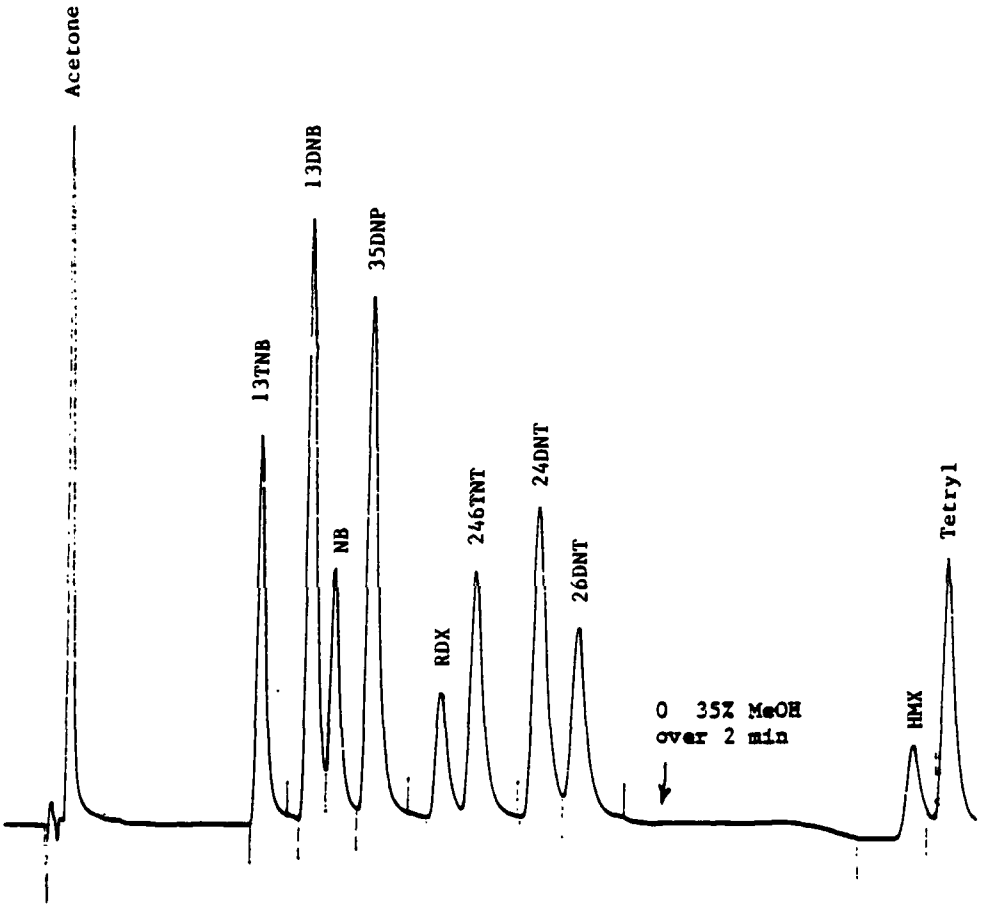
resolved by more polar packings such as CN-types, which are more selective for polar compounds because they consist of a short-chain hydrocarbon terminated by a polar nitrile group. Experiments were conducted to examine the elution behavior of the nitrocompounds on a 4.6-mm by 250-mm, Ultrasphere-CN column.

It was found that an optimum separation of the nitrocompounds could be obtained on an Ultrasphere-CN column using a comparatively weak solvent strength in the mobile phase (<10 percent acetonitrile in phosphate-buffered water at a pH of 3).

Eight of the target nitrocompounds were resolved isocratically using this mobile phase with an increase of solvent strength to 25% methanol required to elute tetryl and HMX. The separation is illustrated in Figure 4-6. Individual standards of each nitrocompound were shot to identify each peak in the chromatogram. A composite standard consisting of the nitrocompounds and PAHs was prepared, and a number of chromatographic conditions were tried to find the optimum separation obtainable on the Ultrasphere-CN column. The CN column did not provide adequate resolution of the PAH compounds.

The experiments on the Ultrasphere-CN column showed that the CN substrate provided unique selectivity suitable for separation of the nitrocompounds; however, it was not suitable for separation of PAHs. The opposite behavior was observed on the Ultrasphere-ODS column. To combine the selectivity of the ODS column for PAHs with the selectivity of the CN column for the nitrocompounds, the elution behavior of the target compounds was examined on the two columns connected in series. The CN column was placed first in the series following the injector, followed by the ODS column.

A number of chromatographic runs were made to optimize the separation of both classes of compounds on this two-column-in-series arrangement. Both acetonitrile and methanol were examined as mobile-phase organic



SOURCE: ESE, 1982.

Figure 4-6

SEPARATION OF NITROCOMPOUNDS ON AN ULTRASPHERE-CN COLUMN

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modifiers. Chromatograms were also run under different column temperatures (65° and 50°C). The final temperature selected was 52°C. The optimum separation of the nitrocompounds was obtained using 30% acetonitrile in phosphate-buffered water at pH = 3 at a flow of 1 ml/minute. Under these conditions, the nitrocompounds were separated isocratically. After 50 minutes, a shallow gradient was initiated to increase the percentage of methanol to a maximum of 90% in several stages. A plot of the percentage-methanol-versus-time is presented in Figure 4-7. The percentage methanol was not increased greater than 90% to avoid any precipitation problems associated with the phosphate-buffered water at high percentages of organic modifier. A 15-minute reequilibration stage at 30% methanol occurred before the next injection. A steeper elution gradient could have been employed; however, this shallow gradient allowed for better resolution of compounds which might appear in the region of intermediate polarity between the polar nitrocompounds and nonpolar PAHs. Also, steeper gradients tend to produce baseline disturbances in the response at 254 nm. These disturbances are likely due to refractive index changes and the slight absorbance of impurities in the methanol at this wavelength. At a column temperature of 52°C, a flow rate of 1 ml/min, and a mobile phase of 30% methanol/70% water, the dual CN/ODS column system produced 23,400 plates as measured from the 24DNT peak (R_t = 28.3 min). At the operating temperature of 52°C, the observed system back pressure was 3,500 psi using a 30% methanol/70% water mobile phase.

Peak identifications were made by injection of each of the individual components under the same elution conditions. Small changes occurred in the resolution of the system over long periods of time. Because the two different columns generally aged at slightly different rates, changes in the retention of each component were compound specific and not unidirectional because of the mixed selectivities involved in using two different stationary phases. Whenever these effects occurred, they were readily noted through observation of the changes in retention times and absorbance ratios of the standards. Up to a point, moderate adjustments

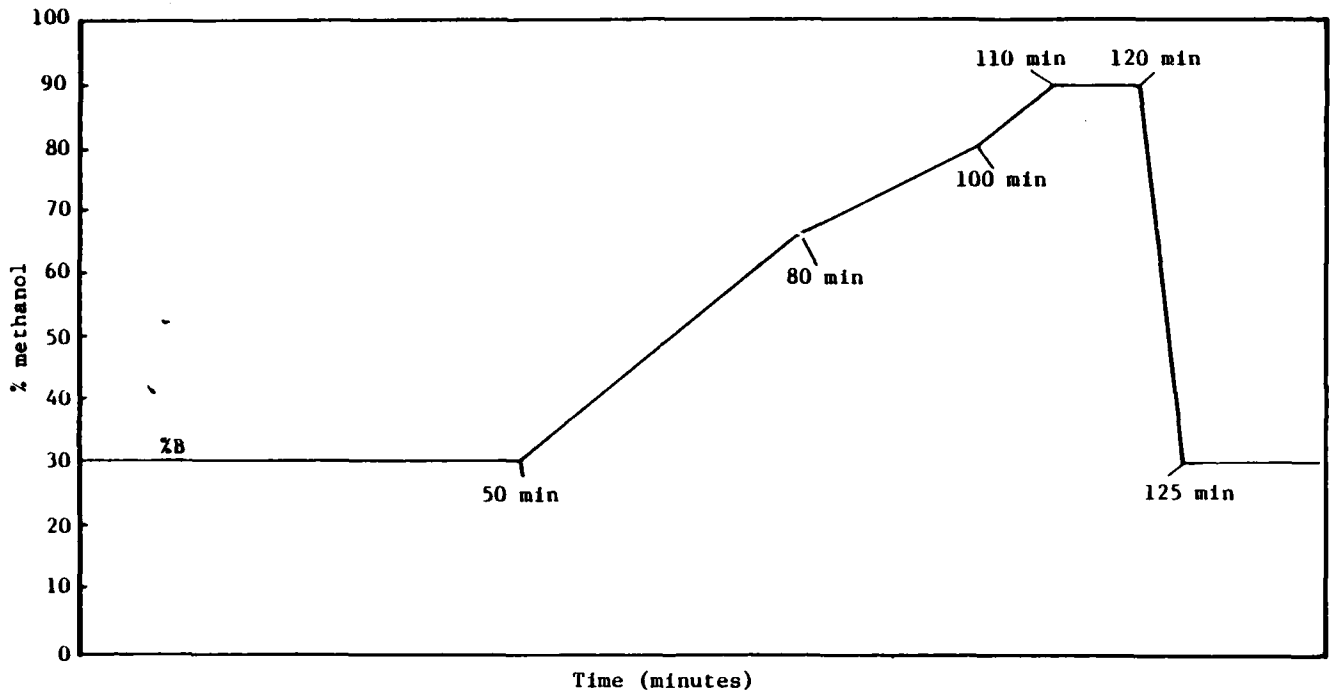

Figure 4-7
ELUTION PROGRAM FOR HPLC SCREEN

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of the elution conditions could be made to compensate for these effects before replacement of one or both the columns was mandated. Experience has indicated that the columns will last 3 to 4 months with constant use at the elevated temperatures of this study.

Representative chromatograms of a standard water sample spiked at the 10-DL level for each analyte using the CN/ODS column system are presented in Figures 4-8 through 4-10. These chromatograms were obtained under the conditions used in documentation of the final method. Component identifications are shown on the chromatograms. The separation illustrated in Figure 4-8 was obtained on the Perkin-Elmer LC-75. The discontinuity noted in the middle of the chromatogram is due to the wavelength change from 230 to 280 nm. The separations obtained with the Altex Model 153 fixed-wavelength detector at 254 nm and the Perkin-Elmer 650-S fluorescence spectrometer, respectively, are presented in Figures 4-9 and 4-10. Only the PAH region is shown in Figure 4-10 because the nitrocompounds do not fluoresce.

Task 3--Development of an Extraction and Cleanup Procedure

The purpose of this task was the development of the wet-chemical methodology to provide a suitable extract for HPLC analysis.

Initial experiments involved spiking a composite nitrocompound standard into organic-free water. Separate water samples were spiked with a composite PAH standard and a standard consisting of both PAHs and nitrocompounds combined. The reason for spiking with three different standards was to check for possible interactions between PAHs and nitrocompounds during the sample workup. During preparation of the composite standard of the nitrocompounds and PAHs, a yellow precipitate was noted in standards dissolved in 100% acetonitrile. This precipitate, as learned later, was due to a reaction between the nitrocompounds and PAHs to give stable charge-transfer complexes. The formation of these complexes is well-known in the literature and can be avoided by the addition of polar substances such as water to the medium.

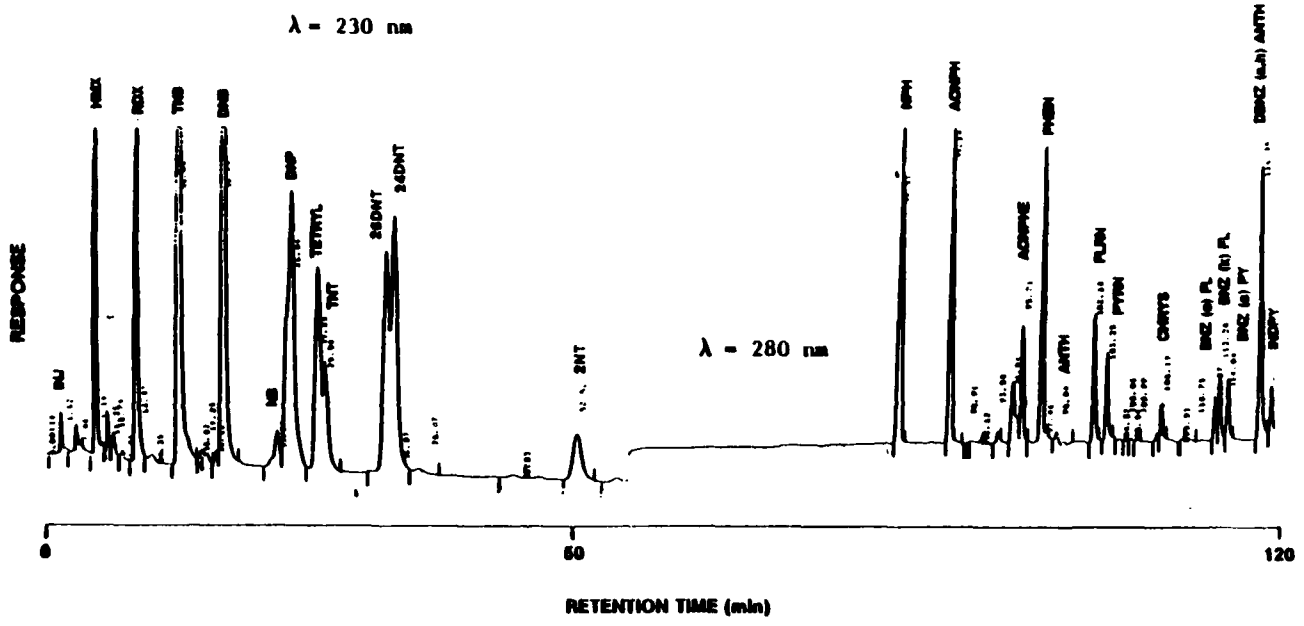

Figure 4-8
HPLC CHROMATOGRAM OF A STANDARD WATER SAMPLE SPIKED AT THE 10X LEVEL USING A PERKIN-ELMER LC-75

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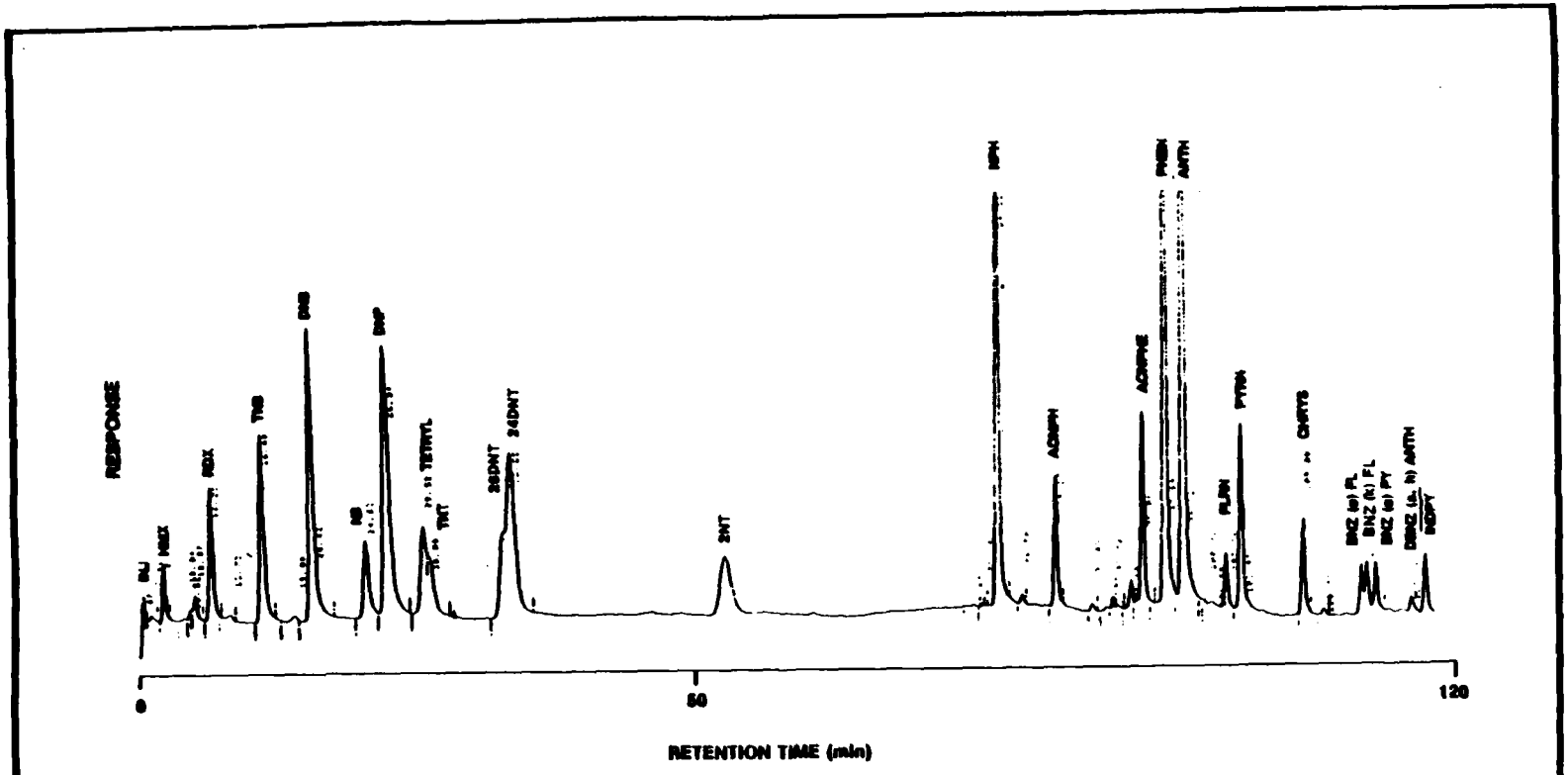

Figure 4-9
HPLC CHROMATOGRAM OF A STANDARD WATER SAMPLE SPIKED AT THE 10X LEVEL USING A 254-nm DETECTOR

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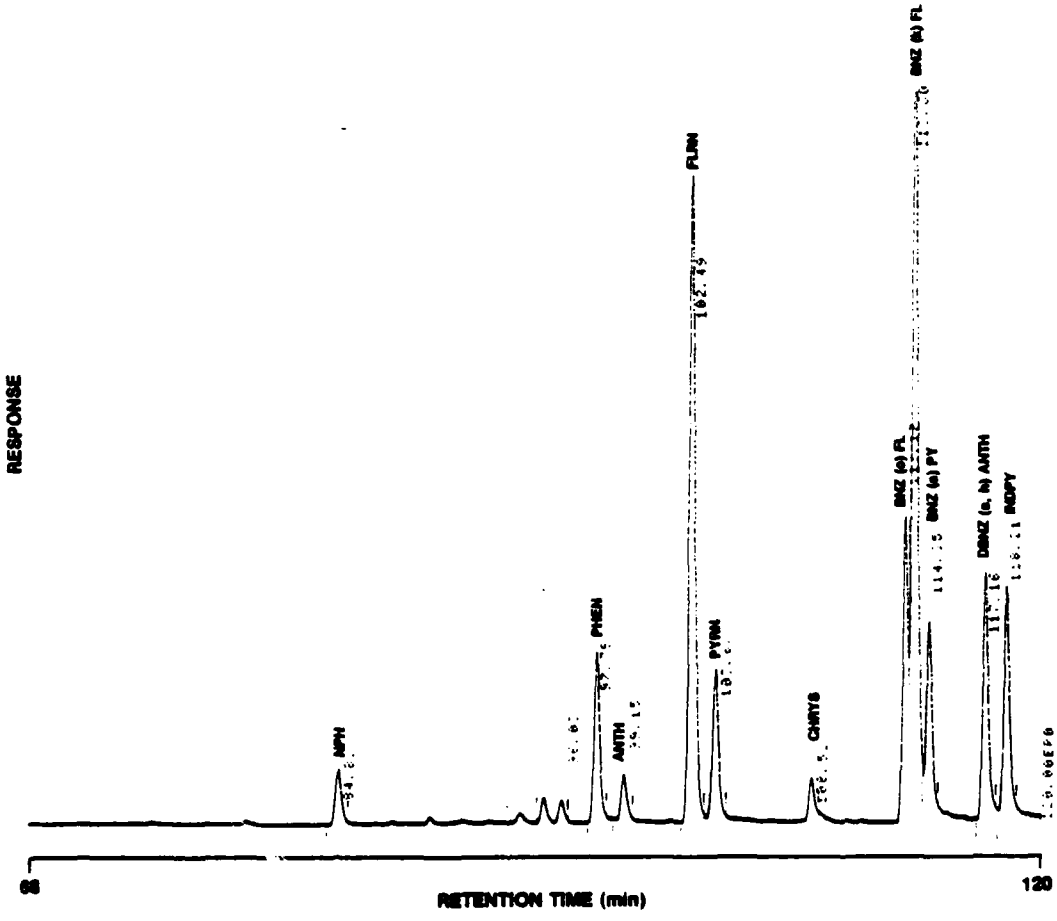

Excitation λ = 290 nm
Emission λ = Filter (>350 nm)

Figure 4-10
HPLC CHROMATOGRAM OF A STANDARD WATER SAMPLE SPIKED AT THE 10-DL LEVEL USING A FLUORESCENCE DETECTOR

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To check for interferences from this complex formation process in the method, the instrumental response of the components in the composite standard was compared to that of individual standards analyzed separately.

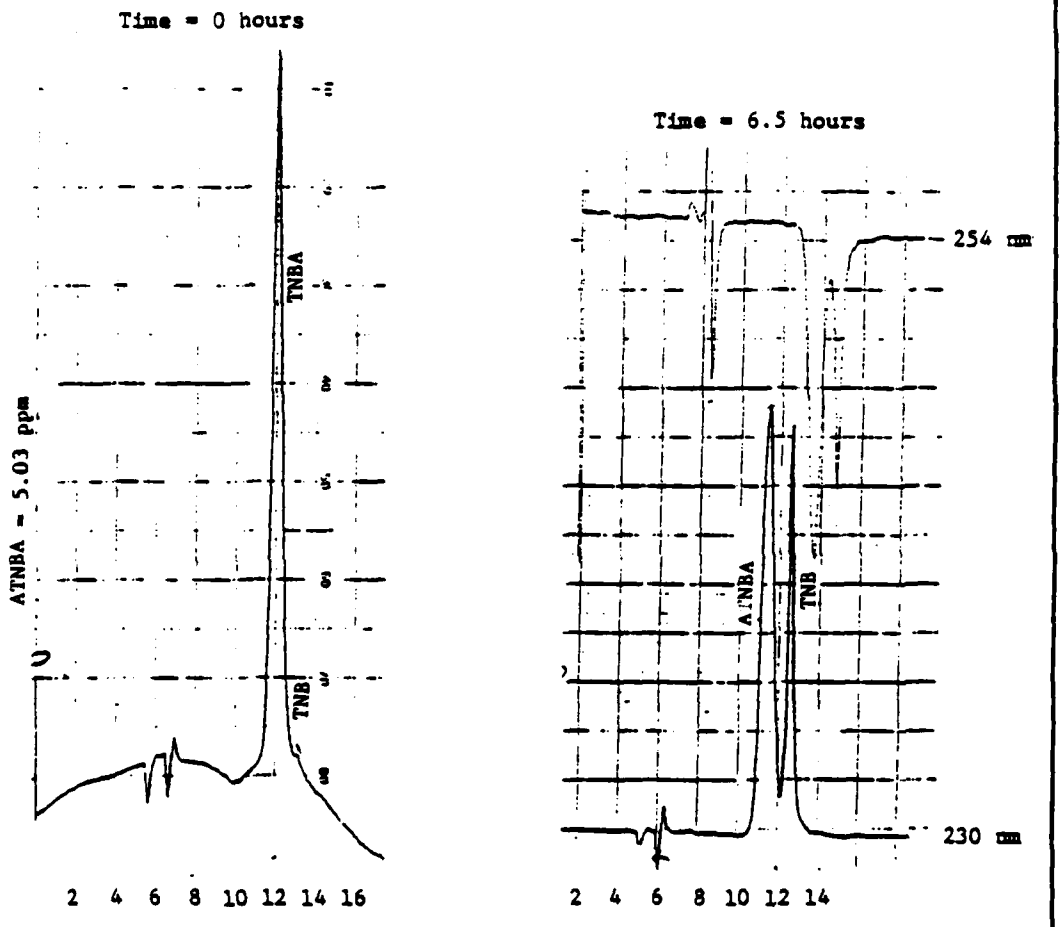
Because individual and mixed standards gave the same sensitivity for all the compounds, it was reasonable to assume that these complexes did not interfere with the method. A further check on possible interaction during sample workup was conducted by performing spiking experiments into standard water with composite (nitro and PAH) spiking solutions and individual spiking solutions. No differences in component recoveries on extraction, concentration, and analysis were noted.

Several experiments were conducted to explore the possibility of inclusion of ATNBA in the screening protocol. This compound is very unstable with respect to hydrolysis, alcoholysis, and oxidation. Spiking experiments were conducted using ATNBA and all nitrocompounds and PAHs together to look for interactions between ATNBA and the other compounds. The sample extracts were concentrated using rotary evaporation techniques. There were no interactions noted and the recovery for ATNBA was approximately 70 percent, which is consistent with the results obtained during the documentation of the ATNBA single-analyte method.

The ATNBA single-analyte method calls for concentration of the extract by rotary evaporation and exchange into acetonitrile to avoid reaction of ATNBA with methanol. A comparison of the recoveries for the nitroaromatic and PAH compounds using K-D as opposed to rotary evaporation techniques showed slightly greater recoveries by the K-D technique. Because of the propensity of ATNBA to decompose in water, inclusion of ATNBA in the screen compounds would require that the extracts remain in acetonitrile and not be diluted with water until immediately before injection. This restriction would have severely limited automation of the screen for a more cost-effective analysis

because samples could not be diluted and placed in the magazine of an autosampler for injection several hours later. The rapid decomposition of ATNBA to TNB during several hours of storage in the mobile phase solvent is illustrated in Figure 4-11. The upper and lower traces in the 6.5-hour chromatogram correspond to detection at 254 and 230 nm, respectively. After discussions with USATHAMA, it was decided to exclude ATNBA from documentation of the HPLC screen method; however, this compound is still detectable by the screen method. ATNBA is well resolved from the other nitrocompounds, and detector ratios were determined for qualitative identification. The retention time for ATNBA by the finalized HPLC method is 14.3 minutes. The chromatograms presented in Figure 4-11 were run under slightly different conditions.

A sample cleanup procedure was developed for the elimination of acidic and highly polar interferents present in natural samples. Commercially available, silica-gel Sep-Pak® cartridges were used for this procedure. These cartridges were used for sample cleanup in the method developed for HMX, RDX, and PETN in soil. Because HMX and RDX were the most polar compounds present in the list of target compounds, the elution solvent used for HMX and RDX was the strongest solvent used to elute the screen compounds (i.e., 50% methanol in methylene chloride). To test the cleanup procedure, samples of water spiked with nitrocompounds and PAHs were extracted with methylene chloride. The extract volume was reduced to approximately 5 ml using a K-D apparatus. The methylene chloride extract was passed through a silica-gel Sep-Pak®, and the effluent collected. The Sep-Pak® was then eluted with 5 ml of 50% methanol in methylene chloride. Because PAHs are not retained by the Sep-Pak® on elution with methylene chloride, they remained in the column effluent. Many nitrocompounds were contained in the 50% methanol/50% methylene chloride fraction. The solvent in each fraction was exchanged three times with acetonitrile. After dilution to approximately 30% acetonitrile in water, the fractions were analyzed by HPLC. The recoveries of PAHs and nitrocompounds were greater than 70 percent and, therefore, sufficient for use in the screening procedure.



SOURCE: ESE, 1982.

Figure 4-11

DECOMPOSITION OF ATNBA IN 50% CH_3CN IN WATER

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4.2.4 Method Documentation

After optimization of the HPLC separation and selection of the extraction and cleanup techniques, it was necessary to select the optimum detector parameters and spiking levels to perform the method documentation and analyze the data obtained.

The absorbance detection wavelengths were selected by a combination of literature review and several experiments using different detector conditions. The two wavelengths selected for monitoring the nitro-substituted munition compounds were 230 and 254 nm. These wavelengths were mandated by the inclusion of RDX and HMX in the target analyte list. Both of these compounds have an absorption spectrum which has a strong end absorbance below 220 nm and a low-oscillator strength maximum at approximately 230 nm, which decreases gradually toward longer wavelengths. The absorbance at 230 nm is approximately six times as intense as the absorbance at 254 nm for HMX and three times for RDX. The absorbance ratios for the other nitrocompounds range from approximately 1 to 3.5 at these wavelengths. Monitoring the absorbance at 254 nm is also suitable for detection of PAHs (254 nm used in EPA Method 610); however, the absorbance of PAHs at 230 nm is low, especially for some larger PAHs. The absorbance of PAHs at 280 nm is much more intense than at 230 nm and provides better sensitivity and selectivity for this class of compounds.

Because the nitrosubstituted munition compounds do not fluoresce, the only criterion involved in selection of the fluorescence conditions was the optimization of detector sensitivity for the PAHs. Ogan et al. (1979) showed that the use of a cutoff filter in fluorescence detectors, which allow the complete emission band of an analyte to be detected, can provide more sensitive detection of PAHs than is possible by selecting a narrow-wavelength interval with a monochromator. For this reason, and because of the cost effectiveness of using filter fluorescence detectors, the emission was measured using a 350-nm cutoff filter with

the emission monochromator set in the zero-order mode. This filter passes all light of wavelengths longer than 350 nm, which includes the emission bands of all PAHs. The excitation wavelength was selected by comparing the sensitivity of each PAH excited at 280, 290, and 300 nm and monitoring the emission at wavelengths longer than 350 nm. The excitation spectral band pass was 10 nm. The response for excitation at 290 nm provided the maximum sensitivity. Excitation at 300 nm showed enhanced response for the later eluting PAHs but also a higher noise level. The earlier eluting PAHs responded less at 300-nm than at 290-nm excitation wavelength.

The detection conditions selected for use are summarized as follows:

Absorbance--230 and 254 nm for the nitrosubstituted munition compounds, and 280 and 254 nm for PAHs.

Fluorescence--Excitation wavelength of 290 nm with a spectral band pass of 10 nm; emission monitored at wavelengths longer than 350 nm.

The spiking levels used in the documentation were selected by consideration of several factors. The desired quantitative detection limit was in the range of 1 to 5 ug/L. Because qualitative identification of the compounds was also desired, the spiking levels had to be such that detector ratios could be generated at as many of the target levels as possible. The sensitivity of the target compounds with respect to each of the three detectors and the dynamic range of the detectors had to be considered in order to provide valid ratios. Several larger PAHs were spiked at levels below the 1-ug/L level because they were not soluble in water at concentrations greater than or near 1 ug/L, and to document the suitability of the developed procedure for detection of PAHs below the requirements of the various regulatory agencies, which often require detection limits below 1 ug/L for PAHs. The analytes were spiked at 0, 0.5 DL, 1 DL, 2 DL, 5 DL, and 10 DL, where DL was the desired target detection limit. The DL for the analytes spiked into standard water and the ratios obtained for each

compound for samples analyzed without and with silica-gel cleanup are presented in Tables 4-8 and 4-9. The uncertainty of the absorbance ratios as measured from peak heights is expressed at the 95-percent confidence interval (α = 0.95) for 95% of the measured population (P = 0.95), considering the finite number of measurements for each ratio.

Dixon and Massey (1969) presented the following confidence interval formula:

$$\bar{x} \pm Ks$$

where: $\bar{x}$ = the mean detector ratio, determined from peak heights;
s = the standard deviation of the measured ratios; and
k = value obtained from a table of confidence interval factors based on the number of measurements made and level of significance desired.

For example, for 20 measurements of the absorbance ratio, k = 2.752 at the 95-percent confidence interval for 95% of the population. The k factor increases for fewer measurements because the uncertainty of the average is a function of the number of times the parameter is measured.

The quantitative detection limits for each analyte as determined by the method of Hubaux and Vos (1970) are presented in Tables 4-8 and 4-9. Also, the lowest levels at which a valid detector ratio can be obtained, the qualitative detection limit, are listed in Tables 4-8 and 4-9. These levels can be considered as the lower limit for qualitative identification of the analyte. A valid detector ratio for an unknown sample component falls within the 95-percent confidence interval for the ratio as obtained from the standard water documentation. Because fluorescence-to-absorbance ratios are dependent on the particular instrumentation used, other laboratories implementing this method must determine these ratios on their own instruments during method certification.

Table 4-8. Absorbance Ratios and Detection Limits of Analyte in Standard Water without Silica-Gel Cleanup

Compound	Detector Wavelength (nm)	Mean Absorbance Ratio* ± 95% CI†	Lowest Concentration for Ratio (ug/L)	Target DL (ug/L)	Documented DL** (ug/L)	Retention Time (minutes)
HMX	230/254	6.6 ± 0.80	4.0	4.0	15	9.2
RDX	230/254	3.0 ± 0.25	2.0	4.0	6	13.0
ATNBA	230/254	2.8	ND††	ND	ND	14.3
135TNB	230/254	3.6 ± 0.29	1.9	3.8	6	16.8
13DNB	230/254	1.9 ± 0.26	2.2	4.4	6	20.8
NB	230/254	0.70	ND	ND	ND	24.8
35DNP	230/254	1.7 ± 0.58	3.6	7.3	11	26.9
Tetryl	230/254	3.14	ND	ND	ND	29.5
246TNT	230/254	2.3 ± 0.43	2.0	4.0	6	29.3
26DNT	230/254	1.5 ± 0.95	2.3	4.6	9	35.0
24DNT	230/254	1.2 ± 0.31	2.0	4.0	6	36.1
12DNT	230/254	0.852				52.5
Naphthalene	280/254	1.6 ± 0.8	4.1	8.2	27	84.5
	254/F1***	21 ± 12	41	8.2		
	280/F1	36 ± 5.0	41	8.2		
Acenaphthylene	280/254	1.3 ± 0.28	6.6	13	22	89.1
Acenaphthene	280/254	0.53 ± 0.13	1.5	4.8	10	95.7
Phenanthrene	280/254	0.35 ± 0.13	1.5	3.0	5	97.5
	254/F1	29 ± 5.9	6.1	3.0		
	280/F1	11 ± 1.6	6.1	3.0		

Table 4-8. Absorbance Ratios and Detection Limits of Analytes in Standard Water without Silica-Gel Cleanup
(Continued, Page 2 of 3)

Compound	Detector Wavelength (nm)	Mean Absorbance Ratio* ± 95% CI†	Lowest Concentration for Ratio (ug/L)	Target DL (ug/L)	Documented DL** (ug/L)	Retention Time (minutes)
Anthracene	280/254	0.0082 ± 0.014	12	2.4	2	98.8
	254/F1	220 ± 56	12	2.4		
	280/F1	1.5 ± 0.31	12	2.4		
Fluoranthene	280/254	2.2 ± 0.64	0.4	0.8	0.9	102.2
	254/F1	0.55 ± 0.16	0.4	0.8		
	280/F1	1.2 ± 0.39	0.4	0.8		
Pyrene	280/254	0.46 ± 0.16	1.9	3.8	8	103.4
	254/F1	7.9 ± 1.5	1.9	3.8		
	280/F1	3.8 ± 1.0	19	3.8		
Chrysene	280/254	0.43 ± 0.15	0.40	0.20	0.4	108.2
	254/F1	15 ± 1.3	1.0	0.20		
	280/F1	6.6 ± 2.5	1.0	0.20		
Benzo(b)fluoranthene	254/280	0.99 ± 0.42	0.21	0.21	2	112.9
	254/F1	0.97 ± 0.27	0.21	0.21		
	280/F1	0.99 ± 0.56	0.21	0.21		
Benzo(k)fluoranthene	280/254	1.3 ± 0.47	0.21	0.41	1	113.3
	254/F1	0.39 ± 0.16	0.21	0.41		
	280/F1	0.50 ± 0.23	0.21	0.41		
Benzo(a)pyrene	280/254	1.6 ± 0.70	0.45	0.45	0.8	114.0
	254/F1	1.3 ± 0.58	0.45	0.45		
	280/F1	2.0 ± 0.83	0.45	0.45		

Table 4-8. Absorbance Ratios and Detection Limits of Analytes in Standard Water without Silica-Gel Cleanup (Continued, Page 3 of 3)

Compound	Detector Wavelength (nm)	Mean Absorbance Ratio* ± 95% CI†	Lowest Concentration for Ratio (ug/L)	Target DL (ug/L)	Documented DL** (ug/L)	Retention Time (minutes)
Dibenzo(a,h)anthracene	280/254	12 ± 6.4	0.75	1.5	ND	116.8
	254/Fl	0.62 ± 0.39	0.75	1.5		
	280/Fl	6.9 ± 2.2	0.75	1.5		
Indeno(1,2,3-cd)pyrene	280/254	0.89 ± 0.27	0.58	0.58	2	117.9
	254/Fl	1.6 ± 0.34	0.58	0.58		
	280/Fl	1.4 ± 0.32	0.58	0.58		

* Determined from peak height measurements.
† CI = Confidence interval (n = 20).
** DL = Detection limit calculated according to Hubaux and Vos (1970).
†† Not determined due to co-elution, interference, or instability problems.
*** Fluorescence measured at wavelengths greater than 350 nm.

Source: ESE, 1982.

Table 4-9. Absorbance ratios and Detection Limits of Analytes in Standard Water with Silica-Gel Cleanup

Compound	Detector Wavelength (nm)	Mean Absorbance Ratio* ± 95% CI†	Lowest Concentration for Ratio (ug/L)	Target DL (ug/L)	Documented DL** (ug/L)	Retention Time (minutes)
HMX	230/254	6.6 ± 2.2	4.0	4.0	11	9.2
RDX	230/254	3.0 ± 0.63	2.0	4.0	8	13.0
135TNB	230/254	3.6 ± 0.79	1.9	3.8	6	16.8
13DNB	230/254	1.9 ± 0.29	2.2	4.4	8	20.8
35DNP	230/254	1.6 ± 0.68	3.6	7.3	8	26.9
246TNT	230/254	2.3 ± 0.76	2.0	4.0	11	29.3
26DNT	230/254	1.7 ± 0.89	2.3	4.6	12	35.0
24DNT	230/254	1.1 ± 0.55	2.0	4.0	7	36.1
Naphthalene	280/254	1.6 ± 0.62	4.1	8.2	33	84.5
	254/Fl††	22 ± 16	41	8.2		
	280/Fl	37 ± 6.0	41	8.2		
Acenaphthylene	280/254	1.4 ± 0.71	6.6	13	35	89.1
Acenaphthene	280/254	0.52 ± 0.47	1.5	4.8	12	95.7
Phenanthrene	280/254	0.35 ± 0.05	1.5	3.0	5	97.5
	254/Fl	31 ± 12	6.1	3.0		
	280/Fl	10 ± 4.7	6.1	3.0		
Anthracene	280/254	0.0084 ± 0.011	12	2.4	5	98.8
	254/Fl	230 ± 72	12	2.4		
	280/Fl	1.9 ± 2.5	12	2.4		

Table 4-9. Absorbance Ratios and Detection Limits of Analytes in Standard Water with Silica-Gel Cleanup (Page 2 of 3)

Compound	Detector Wavelength (nm)	Mean Absorbance Ratio* ± 95% CI†	Lowest Concentration for Ratio (ug/L)	Target DL (ug/L)	Documented DL** (ug/L)	Retention Time (minutes)
Fluoranthene	280/254	2.3 ± 0.56	0.4	0.8	1	102.2
	254/F1	0.53 ± 0.13	0.4	0.8		
	280/F1	1.2 ± 0.30	0.4	0.8		
Pyrene	280/254	0.48 ± 0.17	1.9	3.8	6	103.4
	254/F1	7.8 ± 1.4	7.7	3.8		
	280/F1	3.7 ± 0.97	7.7	3.8		
Chrysene	280/254	0.40 ± 0.046	0.40	0.20	0.5	108.2
	254/F1	16.2 ± 5.9	1.0	0.20		
	280/F1	6.0 ± 5.0	1.0	0.20		
Benzo(b)fluoranthene	280/254	1.0 ± 0.46	0.21	0.21	0.3	112.9
	254/F1	0.94 ± 0.30	0.21	0.21		
	280/F1	0.96 ± 0.38	0.21	0.21		
Benzo(k)fluoranthene	280/254	1.4 ± 0.71	0.41	0.41	0.8	113.3
	254/F1	0.39 ± 0.26	0.21	0.41		
	280/F1	0.53 ± 0.26	0.41	0.41		
Benzo(a)pyrene	280/254	1.8 ± 0.52	0.45	0.45	0.9	114.0
	254/F1	1.3 ± 0.51	0.45	0.45		
	280/F1	2.0 ± 1.2	0.45	0.45		

Table 4-9. Absorbance Ratios and Detection Limits of Analytes in Standard Water with Silica-Gel Cleanup (Page 3 of 3)

Compound	Detector Wavelength (nm)	Mean Absorbance Ratio* ± 95% CI†	Lowest Concentration for Ratio (ug/L)	Target DL (ug/L)	Documented DL** (ug/L)	Retention Time (minutes)
Dibenzo(a,h)anthracene	280/254	13 ± 3.7	1.5	1.5	ND***	116.8
	254/F1	0.61 ± 0.28	1.5	1.5		
	280/F1	7.7 ± 2.9	1.5	1.5		
Indeno(1,2,3-cd)pyrene	280/254	0.88 ± 0.38	0.58	0.58	2	117.9
	254/F1	1.6 ± 0.77	0.58	0.58		
	280/F1	1.4 ± 1.0	0.58	0.58		

* Determined from peak height measurements.
† CI = Confidence interval (n = 20).
** DL = Detection limit calculated according to Hubaux and Vos (1970).
†† Fluorescence measured at wavelengths greater than 350 nm.
*** ND = Not determined due to interference problems.

Source: ESE, 1982.

In many instances, the qualitative detection limit extends to the 0.5X level; however, for some of the analytes, the qualitative detection limit for the absorbance-to-fluorescence ratio is considerably higher than the targeted quantitative detection limit. This effect is exhibited in the nitrocompounds, acenaphthylene, acenaphthene, and naphthalene, because these compounds exhibit weak or nonexistent fluorescence. In all but one of these cases, the absorbance ratio is useful for qualitative identification to the 0.5-DL level and the 1-DL level for HMX. The only exception is anthracene, which exhibits weak absorbance at 280 nm and also weak fluorescence; therefore, the lowest valid ratio concentration for anthracene is closer to the 5-DL level.

In an attempt to obtain the most consistent ratios possible, an internal standard was used in the measurement and calculation of the ratios. A small aliquot of a standard solution of 2-nitrotoluene (2NT) was added to the final extract before HPLC analysis. The rationale for this approach was based on minimizing the variance of each detector relative to each other by normalizing the peak height of each analyte to the peak height of 2NT on the same detector. Ratios for each analyte were calculated using the normalized responses as follows:

$$\text{Ratio} = \frac{\left(\frac{\text{Peak height of analyte on Detector 1}}{\text{Peak height of 2NT on Detector 1}}\right)}{\left(\frac{\text{Peak height of analyte on Detector 2}}{\text{Peak height of 2NT on Detector 2}}\right)}$$

However, this procedure did not improve the long-term variance of the ratios; in fact, the variance of the ratios approximately doubled due to the compounding of the variances of the measurement of the ratio for two compounds as opposed to one. Another factor that led to the abandonment of this approach is the possibility of interferents co-eluting with the

2NT in actual samples which would affect the selectivity of qualitative analysis.

During the documentation of the method, three different analysts were employed to check the ruggedness of the extraction and cleanup procedure. Each analyst conducted at least 1 day of spiking experiments completely independent of the other two analysts. It was found that a critical step occurs in the splitting of the original sample extract into two fractions, one that will be analyzed directly and one that will be cleaned by silica gel. It was important to ensure that the sample was well-mixed when dividing the extract into two portions; otherwise, unequal recoveries were obtained for the two fractions.

The full documentation data for standard and natural waters are presented in the HPLC screen method in Appendix C.

The detector ratios and 95-percent confidence intervals were determined in standard water for the silica-gel-cleaned and uncleaned extracts. As a check of the validity of the natural water ratios as qualitative identifiers, detector ratios were obtained for these samples. A typical set of ratios obtained at the 5-DL spiking level during the natural water documentation and the ratios obtained in the standard water documentation are presented in Table 4-10. In all cases, the natural water ratios fall within the 95-percent confidence interval specified for qualitative matching of the ratios. Quantitative documentation in natural water was also performed for all analytes; the results of these experiments are presented in Appendix C.

Quantitative documentation on dibenzo(a,h)anthracene in standard and natural water presented a problem. Variable recoveries were noted in the spiked sample data which led to an unacceptably high Hubaux and Vos (1970) detection limit of 16 ug/L, which was greater than the highest spiked standard (15 ug/L). The reason for this variability is unclear; however, it is probably not due to co-eluting interferent peaks because

Table 4-10. Comparison of Ratios for a Natural Water Sample versus Standard Ratio Criteria

	Detector Wavelengths (nm)	Standard Water Absorbance Ratio ± 95% CI*	Natural Water Absorbance Ratio†
HMX	230/254	6.6 ± 0.80	6.8
RDX	230/254	3.0 ± 0.25	2.9
135TNB	230/254	3.6 ± 0.29	3.5
13DNB	230/254	1.9 ± 0.26	1.9
35DNP	230/254	1.7 ± 0.58	1.8
246TNT	230/254	2.3 ± 0.43	2.3
26DNT	230/254	1.5 ± 0.95	1.8
24DNT	230/254	1.2 ± 0.31	1.2
Naphthalene	280/254	1.6 ± 0.8	1.5
	254/F1**	21 ± 12	23
	280/F1	36 ± 5.0	35
Acenaphthylene	280/254	1.3 ± 0.28	1.2
Phenanthrene	280/254	0.35 ± 0.13	0.34
	254/F1	29 ± 5.9	31
	280/F1	11 ± 1.6	11
Anthracene	280/254	0.0082 ± 0.014	0.006
	254/F1	220 ± 56	223
	280/F1	1.5 ± 0.31	1.3
Fluoranthene	280/254	2.2 ± 0.64	2.4
	254/F1	0.55 ± 0.16	0.57
	280/F1	1.2 ± 0.39	1.4

Table 4-10. Comparison of Ratios for a Natural Water Sample versus Standard Ratio Criteria (Continued, Page 2 of 2)

	Detector Wavelengths (nm)	Standard Water Absorbance Ratio ± 95% CI*	Natural Water Absorbance Ratio†
Pyrene	280/254	0.46 ± 0.16	0.44
	254/F1	7.9 ± 1.5	8.9
	280/F1	3.8 ± 1.0	3.9
Chrysene	280/254	0.43 ± 0.15	0.43
	254/F1	15 ± 1.3	16
	280/F1	6.6 ± 2.5	7.1
Benzo(b)fluoranthene	280/254	0.99 ± 0.42	0.89
	254/F1	0.097 ± 0.27	1.1
	280/F1	0.99 ± 0.56	0.96
Benzo(k)fluoranthene	280/254	1.3 ± 0.47	1.2
	254/F1	0.39 ± 0.16	0.40
	280/F1	0.50 ± 0.23	0.46
Benzo(a)pyrene	280/254	1.6 ± 0.70	1.8
	254/F1	1.3 ± 0.58	1.2
	280/F1	2.0 ± 0.83	2.2
Indeno(1,2,3-cd)pyrene	280/254	0.89 ± 0.27	0.98
	254/F1	1.6 ± 0.34	1.4
	280/F1	1.4 ± 0.32	1.4

* Confidence interval (n = 20); rates determined by peak heights.
† Natural water ratio determined from a 5-DL-level spiked sample not cleaned by silica gel.
** Greater than 350 nm.

Source: ESE, 1982.

none were noted in the matrix blanks and the detector ratios did not display a wide variability.

Two analytes, nitrobenzene and tetryl, were not fully documented in standard and natural water because of co-elution problems under the chromatographic conditions employed. When the CN-ODS column system was first investigated for the separation of the nitroaromatic compounds, both tetryl and nitrobenzene were resolved from any other analytes. As the columns aged with constant use at elevated temperatures, the retention times of nitrobenzene and tetryl were noticed to undergo gradual shifts and to overlap. The overlap between these peaks prevented adequate quantitation and, thus, they were not included in the documentation spiking mixture. Midway through the standard water documentation, a change in the Ultrasphere-ODS column was necessitated by poor column performance as indicated by poorly resolved peaks Upon substitution of a new ODS column, the retention time for tetryl substantially shifted and no longer overlapped with nitrobenzene. The chromatograms are illustrated in Figures 4-8 and 4-9. No other major changes in elution order for the other components were noted when the ODS column was replaced, and restandardization of the column system due to small changes in retention times was performed.

Documentation of this HPLC screen required a considerable amount of data reduction because more than 20 compounds were documented using both silica-gel-cleaned samples and uncleaned samples. Data were generated by three different detectors for standard and natural water. Detector ratios were calculated for the cleaned and uncleaned standard water samples for each spiking experiment. To examine the use of an internal standard for calculation of the ratios used in the qualitative identifications, ratios were calculated using the response of ONT to normalize the response for each compound. Unnormalized response ratios were also calculated. To facilitate data calculation, a computer program was generated for use on the Spectra Physics SP4100 computing integrator.

The program was written in BASIC and can be readily modified for use with other computers.

A listing of the computer program, an example of the data output, and a brief explanation of the salient details are presented in Appendix D.

4.2.5 Suggestions for Further Experiments

This HPLC screening procedure can be readily expanded to include a larger number of analytes. The gradient employed in the elution of PAHs is rather shallow and should provide excellent resolution of compounds in the region of intermediate polarity between the nitrocompounds and PAHs. The type of compounds expected in this region includes benzenoid structures with nonpolar to moderately polar substituents such as the xylenes, cresols, halogenated benzenoids, and benzene. Because the extraction procedure has been shown to be effective for a wide range of compounds, it is expected that no procedural modifications would be necessary to include many more compounds in the screen. A large data base of qualitative identification parameters, such as ratios and retention times, could be readily generated by analysis of individual standards of compounds of interest. The usual USATHAMA qualitative documentation (1 day of spiking at six levels) should be sufficient to include a compound on the list of compounds for screening.

Further selectivity for the qualitative analysis can be obtained through the use of other types of detectors in conjunction with those already in use. Reductive electrochemical detection (HPLC/EC) is a very sensitive technique for nitrosubstituted munition compounds. The isocratic elution of the nitrocompounds, a requirement of HPLC/EC, is already satisfied by the separation scheme. Because a limited number of compounds are detected by this technique, added selectivity can be achieved through the use of this detector in conjunction with the absorbance detectors to generate compound-specific ratios. Recently, detectors which employ both of these detectors in a single instrument have become commercially available.

Because of the general lack of fine structure of absorbance and fluorescence spectra, it is difficult to distinguish between structurally similar PAHs. Naphthalene and the methyl-substituted naphthalenes have similar spectra and should not exhibit different retention and spectroscopic behavior. VoDinh et al. (1978) developed a technique to obtain the synchronous spectra of PAHs in bulk solutions without separation. VoDinh et al. were able to distinguish between naphthalene and 2-methylnaphthalene, which display synchronous peaks at 323 and 327 nm, respectively. Using standard spectroscopic techniques, the spectra of these compounds should not be different, and the compounds could not be distinguished from one another. Synchronous spectra, as applied to HPLC peaks, have not appeared in the literature and would be a powerful adjunct to the qualitative analysis; however, stopped-flow spectra would have to be obtained and would be less efficient but more selective than this approach.

Because this method has a chromatographic run time of nearly 2 hours, further efforts could be directed toward reducing the time by the use of 3-um columns, which should increase or maintain resolution with a shorter column length and concomitant reduction in time. Another alternative is to employ a solvent optimization routine to obtain separation on shorter dual-column systems.

4.2.6 Cost Effectiveness

The compounds determined by the HPLC screening procedure may also be determined using separate GC, HPLC, and GC/MS techniques. Comparison of the HPLC screening procedure to the individual techniques reveals several advantages for the screening procedure. The primary advantage is the reduction in manhour effort and corresponding labor costs. Secondly, only one set of HPLC instrumentation is required for analysis of all analytes as compared to the GC, HPLC, and GC/MS required to perform the individual analyses. In addition, the HPLC screening technique presented in this document can provide qualitative as well as quantitative information.

The chief disadvantage of the HPLC screening method as presented here is the required availability of several UV and fluorescence detectors and the long analysis times. However, the method as presented was not difficult to set up from commercially available equipment, and little interfacing problems were encountered. The new second generation of UV detectors with automated wavelength scanning and absorbance ratioing can be adapted to this technique and can significantly expand its utility and reduce the complexity of the instrumentation. A cost comparison of the HPLC screening analysis of PAHs and nitroaromatics compared to the analysis for these analytes by separate GC, GC/MS, and HPLC techniques is presented in Table 4-11.

For purposes of comparison, four cost elements are included for each method: extraction, analysis, data reduction, and other direct costs. The labor rates are assumed to be equal for all analyses and the appropriate other direct costs are included for each analysis. Other direct costs included laboratory charges for expendable supplies and GC/MS instrument charges. A batch of ten samples is used for comparison, and all analytes included in the HPLC screening procedure are assumed present in each sample. Analysis by the separate procedures requires the performance of three analytical methods, i.e., HPLC for RDX, HMX, and PETN; GC/EC for the nitroaromatics 246TNT, 24DNT, 26DNT, 13DNB, 135TNB, and NB; and either HPLC by EPA Method 610 or GC/MS by EPA 625 for the PAHs. The data indicate that the HPLC screening procedure requires approximately 50% less expenditure of manhours and costs as compared to the combined individual procedures.

The investment in hardware required to perform the HPLC screen method as presented here can be substantially reduced if no qualitative information is desired (e.g., absorbance ratios). In this case, the basic separation technology with a fixed-wavelength UV detector can be applied to analyze the majority of the test compounds if sacrifices in sensitivity for some of the compounds can be tolerated.

Table 4-11. Cost Comparison Between HPLC Screening and Multiple Individual Procedures

Cost Element	Costs in Manhours/Dollars ($)*				
	1 HPLC Screen (All Analytes)	2 HPLC Method (RDX, PETN, HMX)	3 GC/EC Method (Nitroaromatics)	4 HPLC Method 610 (PAHs)	5 GC/MS Method 625 (PAHs)
Extraction	10/$80	10/$80	6/$48	12/$96	10/$80
Analysis	30/$240	8/$64	10/$80	15/$120	24/$192
Data Reduction	8/$64	8/$64	8/$64	8/$64	8/$64
Other Costs†	$240	$130	$120	$175	$260
Total Manhours	48	26	24	35	42
Total Dollars	$624	$338	$312	$455	$596

Total Dollars and Manhours by Individual Methods 2, 3, and 4 = $1,105, 85 manhours
Total Dollars and Manhours by Individual Methods 2, 3, and 5 = $1,246, 92 manhours

* Assumes $8 per hour direct labor rate (excluding overhead).
† Includes expendable supplies at $5 per manhour; GC/MS instrument charges at $25 per instrument hour for 2 hours.

Source: ESE, 1982.

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